

**DICLOFENAC IN-VITRO ANTIARTHRITIC ACTIVITY - REVIEW**

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ABSTRACT

Diclofenac is a nonsteroidal anti-inflammatory drug (NSAID) of the phenylacetic acid class with anti-inflammatory, analgesic, and antipyretic properties. Contrary to the action of many traditional NSAIDs, diclofenac inhibits cyclooxygenase (COX)-2 enzyme with greater potency than it does COX-1. Similar to other NSAIDs, diclofenac is associated with serious dose-dependent gastrointestinal, cardiovascular, and renal adverse effects. This study aims to evaluate the diclofenac antiarthritic activity. The diclofenac sodium significant anti-arthritis activity at 20- 100 µg/ml by protein denaturation inhibition. 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. The maximum activity is exhibited by the diclofenac at a concentration of 80µg/ml and 100 ug/ml . From the study conducted it can be concluded that diclofenac sodium can be used in the management of arthritis.

Keywords: Diclofenac , arthritis , Nsaids.

INTRODUCTION

All over the world plants were used as main sources of medicine for human kind. The rise of modern western medicine was initially accompanied by a decline in the practice of herbalism in all cultures and we started believing that synthetic chemical were the best medicines to treat illness and cure disease (Singh M. D.et al., 1992). Plant based medicines have been used by mankind since time immemorial. According to the report of World Health Organization (WHO), over 80 % of the world population relies on the traditional system of medicine, largely plant based, to meet their primary health care (Gupta Pet al., 2003).

Medicinal plants have been used in the form of folklore medicine or traditional medicine and ethnic medicine (Indian Herbal Pharmacopoeia, 1999). Herbs are the general way to support and tone the body's system. As with any therapy, one should work with health care provider to diagnose the problem before commencement of treatment (Acharya D et al.,1998). Herbs are used as dried extracts (capsule, powder, teas), glycerite extract (glycerin extracts), or tinctures (alcohol extract) with various herbal preparations traditional systems had developed a science which deals with major disorder. However scientific evaluation of these treatments is the

need of us to reinforce the trust of scientist and validate the traditional systems of medication (Brahmachari UN et al., 2001).

Herbal medicines

In the last few decades there has been an exponential growth in the field of herbal medicine. It is getting popularized in developing and developed countries due to its natural origin and lesser side effects. In ancient times, Vaidya used to treat patients on individual basis, and prepare drug according to the requirement of the patient. Herbalism is a traditional medicinal or folk medicine practice based on the use of plants and plant extracts (Shrivastava D et al.,2008).

Uses of herbal drugs in medicines

In India, herbal drugs are very important part of Indian Medicine system (Ayurveda) which is an ancient and conventional system. Moreover, our culture is rich in herbal drugs in that way causing a high incidence of their self-medication, as also these drugs are sold openly (Sabina EP et al., 2008).

History of arthritis

Arthritis has a severe impact on people of all ages and has been known to mankind since ancient times. Little was known of the diseases, except its symptoms and signs. Rheumatoid arthritis for example can be traced back to dinosaurs and prehistoric man (Emery P et al., 2002).The first published research reports are a few centuries old. According to a book by Bruce M. Rothschild, “The Complete Dinosaur”, only a small portion of dinosaurs actually suffered anything resembling human arthritis. On the other hand, fossil records show evidence that other forms of arthritis did affect dinosaurs, specifically gout. A detailed examination by Rothschild of the bones of a Tyrannosaurus Rex showed the distinctive holes found in the bones of gout patients (Taylor DJ et al., 1994). In 1715 William Musgrave published the second edition of his most important medical work De arthritide symptomatic which concerned arthritis and its effects. Blood tests and X-rays of the affected joints often are performed to make the diagnosis. Screening blood tests are indicated if certain arthritide are suspected. These might include: rheumatoid factor, antinuclear factor (ANF), extractable nuclear antigen, and specific antibodies (Mandal A et al., 1994).

Epidemiology

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 more common in women than men at all ages and affects all races, ethnic groups and cultures. In the United States a CDC survey based on data from 2007–2009 showed 22.2% (49.9 million) of adults aged ≥ 18 years had self-reported doctor-diagnosed arthritis, and 9.4% (21.1 million or 42.4% of those with arthritis) had arthritis-attributable activity limitation(AAAL) With an aging population this number is expected to increase (Combe B et al., 2001).

RA arises from an immunologic reaction, and there is speculation that it is in response to a genetic or infectious antigen. Risk factors associated with the development of RA include Female gender (3:1 females to males).

- Increasing age (peak onset 35 to 50 years of age)
- Current tobacco smoking. Studies have identified a direct relationship between tobacco use and RA disease severity. Tobacco users also have an increased risk of pulmonary manifestations of RA. This risk is reduced when a patient has remained tobacco-free for at least 10 years.
- Family history of RA. Genetic studies demonstrate a strong correlation between RA.
- Potential environmental exposures. The number of RA cases has increased during industrialization, although a specific link to environmental factors has not been determined.
- Oral contraceptive use and high ingestion of vitamin D and tea are associated with a decreased risk of RA.

Types of arthritis

There are over 100 types of arthritis. Here is a description of some primary common ones, together with the causes:

Osteoarthritis- Osteoarthritis is a joint disease that mostly affects cartilage. Cartilage is the slippery tissue that covers the ends of bones in a joint. Healthy cartilage allows bones to glide over each other. It also helps absorb the shock of movement. In osteoarthritis, the top layer of cartilage breaks down and wears away. This allows bones under the cartilage to rub together. The rubbing causes pain, swelling, and loss of motion of the joint. Osteoarthritis occurs most often in older people. Younger people sometimes get osteoarthritis, primarily from joint injuries.

Rheumatoid arthritis - Rheumatoid arthritis is an autoimmune disease in which the normal immune response is directed against an individual's own tissue, including the joints, tendons, and bones, resulting in inflammation and destruction of these tissues. The cause of rheumatoid arthritis is not known. Yet, new research is giving us a better idea of what makes the immune system attack the body and create inflammation. In RA, the focus of the inflammation is in the synovium, the tissue that lines the joint. Immune cells release inflammation-causing chemicals. These chemicals can damage cartilage and bone.

Infectious arthritis (Septic arthritis) - Septic, or infectious, arthritis is infection of one or more joints by microorganisms. Normally, the joint is lubricated with a small amount of fluid that is referred to as synovial fluid or joint fluid. The normal joint fluid is sterile and, if removed and cultured in the laboratory, no microbes will be found. With septic arthritis, microbes are identifiable in an affected joint fluid. Most commonly, septic arthritis affects a single joint, but occasionally more joints are involved.

Juvenile Rheumatoid arthritis (JRA) –Juvenile rheumatoid arthritis is a chronic, autoimmune, inflammatory joint disease. It is the most common rheumatic disease in children and adolescents. It is defined as ‘persistent arthritis

of unknown etiology that begins before the age of 1ars and persists for at least 6 weeks'. It is diagnosed after excluding other

infections may trigger the condition in genetically susceptible children. However, it is unusual for more than one child in a family to have arthritis.

Pathophysiology of arthritis

Most types of arthritis are caused by many factors acting together. Genetic make-up had possibility likely to develop certain disorders. A variety of 'external' factors could also increase this risk further but for many conditions there's a strong element of chance. It can start suddenly without any obvious cause, and at any age(Vincenti MP et al., 1993). Sometimes something in your lifestyle or medical history, such as smoking, could increase your risk further if you're already susceptible to the condition. The ligaments are like elastic bands, while they keep the bones in place our muscles relax or contract to make the joint move. Cartilage covers the bone surface to stop the two bones from rubbing directly against each other. The covering of cartilage allows the joint to work smoothly and painlessly (Hasty KA et al., 1990).

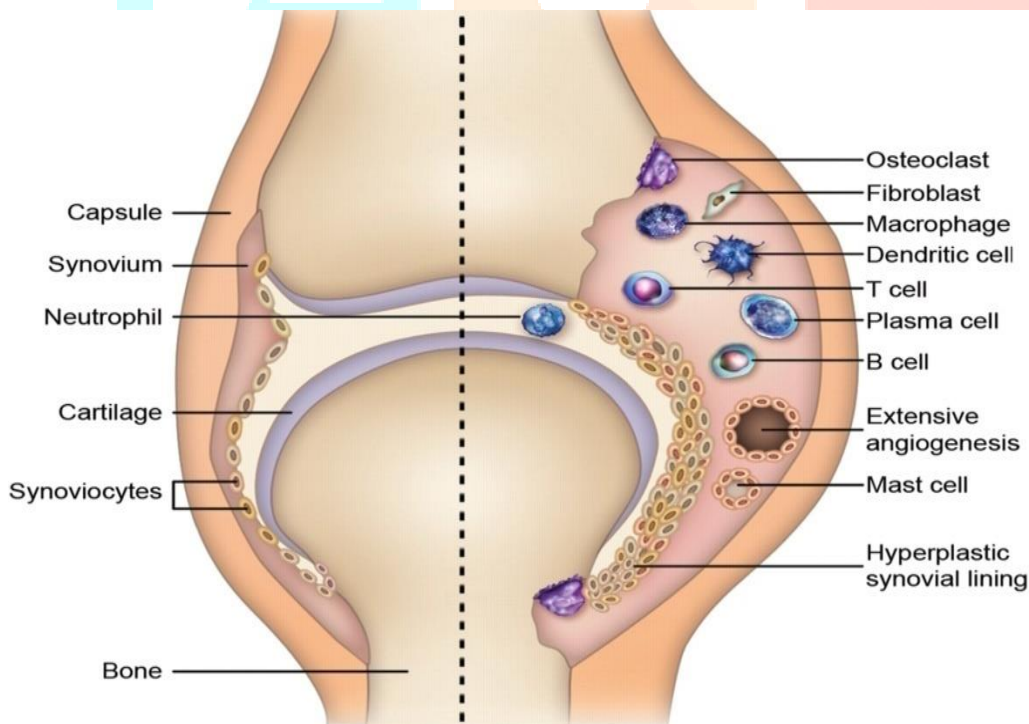


Figure 1: Diagram of Normal Knee and Arthritic Knee

A capsule surrounds the joint. The space within the joint - the joint cavity - has synovial fluid. Synovial fluid nourishes the joint and the cartilage. The synovial fluid is produced by the synovium (synovial membrane) which lines the joint cavity. If you have.

Although the detailed pathogenesis of rheumatoid arthritis is largely unknown, it appears to be an autoimmune disease driven primarily by activated T cells, giving rise to T cell-derived cytokines, such as IL-1 and TNF- α .

Activation of B cells and the humoral response also are evident, although most of the antibodies generated are IgGs of unknown specificity, apparently elicited by polyclonal activation of B cells rather than from a response to a specific antigen .

Pathogenesis of RA:

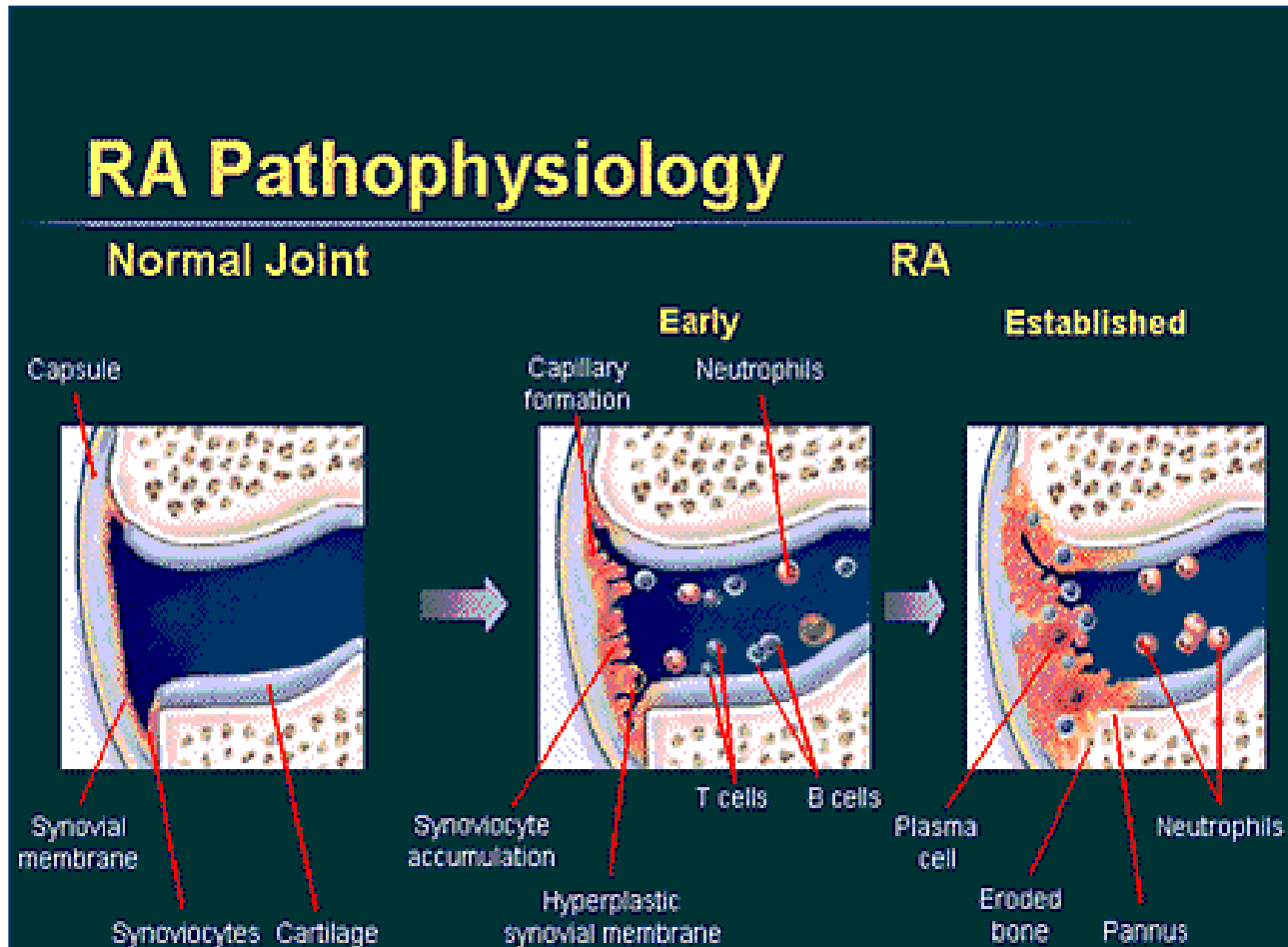


Figure 2: Pathophysiology of RA. It shows Inflammation of Synovium in RA

WBC of immune system moves into joint



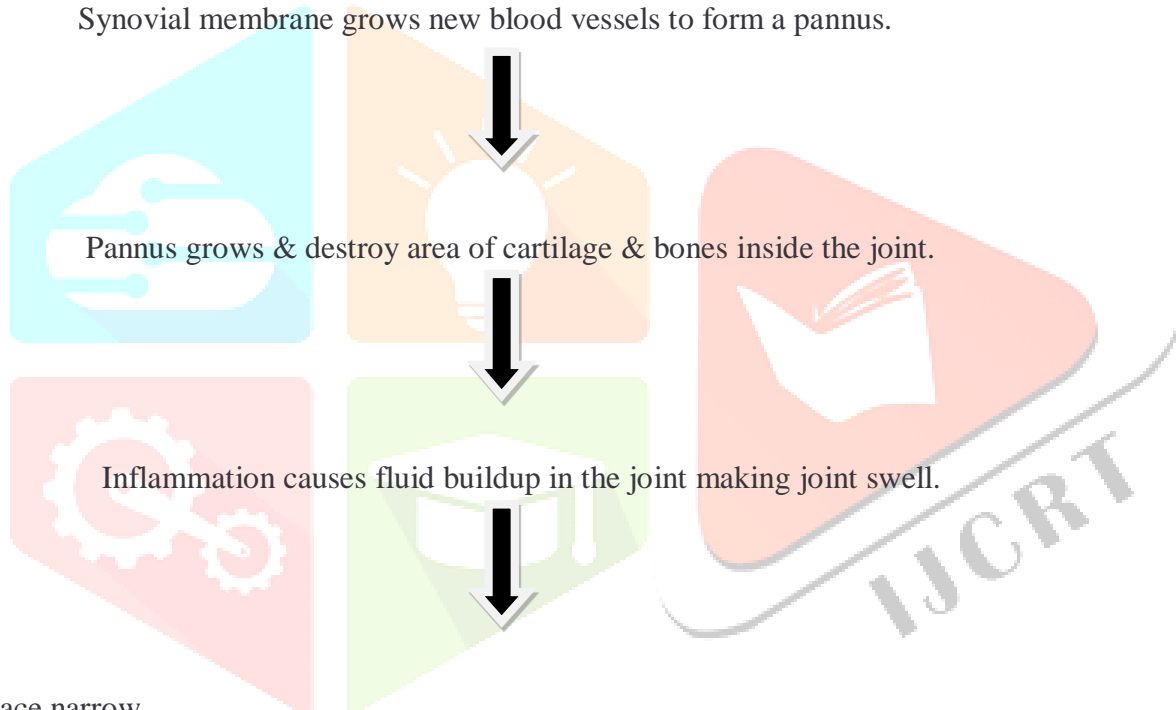
Release cytokine which attack the cell of synovial membrane.



Release other destructive substance (TNF- α , IL1, IL6).



Synovial membrane grows new blood vessels to form a pannus.



Pannus grows & destroy area of cartilage & bones inside the joint.

Inflammation causes fluid buildup in the joint making joint swell.

Joint space narrow

Causative and risk factor:

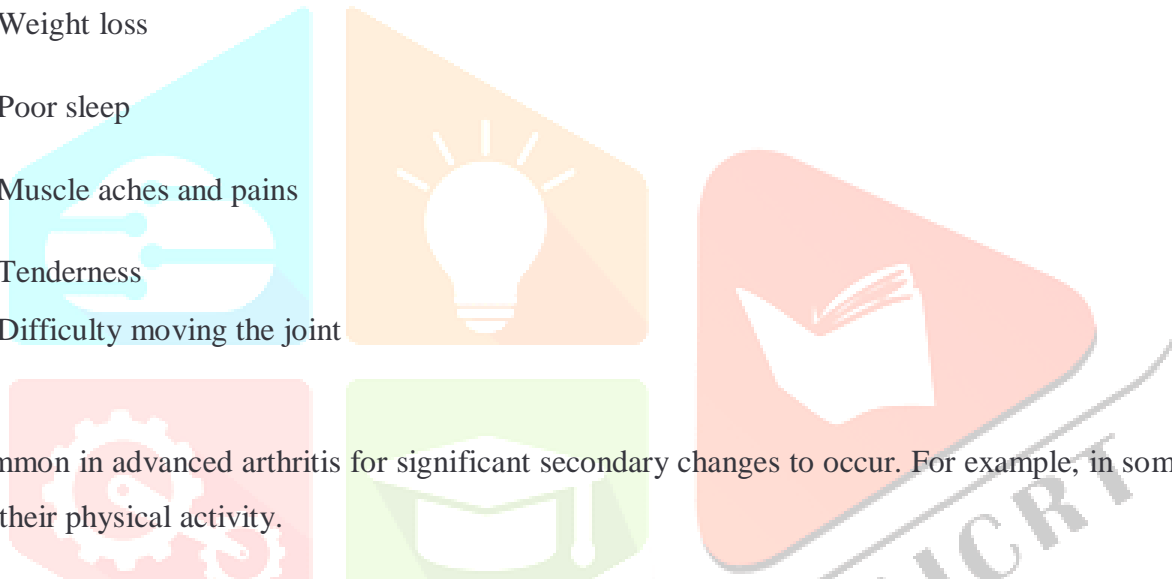
- Genetic makeup had possibility likely to develop certain disorder.
- Obesity.
- Greater bone density.
- Repetitive joint overuse.
- Joint injury joint dysplasia repetitive excessive joint torsion.
- Crystal deposition.
- Neuromuscular dysfunction.
- Penetration of subchondral bone.
- Joint abrasion.

Abnormalities of the articular cartilage may contribute to the degeneration of the joint by causing stress or parts of the articular surface and joint instability.

Signs and symptoms of arthritis:

There is many type of arthritis, the common symptoms for all arthritis disorders include varied levels of pain, swelling, joint stiffness and sometimes a constant pain around the joints. Arthritic disorders like lupus and rheumatoid can also affect other organs in the body with a variety of symptoms.

- Inability to use the hand or walk
- Malaise and a feeling of tiredness
- Fever
- Weight loss
- Poor sleep
- Muscle aches and pains
- Tenderness
- Difficulty moving the joint



It is common in advanced arthritis for significant secondary changes to occur. For example, in someone who has limited their physical activity.

Diclofenac:

It is most commonly used DMARD. It is effective in reducing signs and symptoms of rheumatoid arthritis and slows down damage to the joint. Results can be seen in 6 to 8 weeks. Other DMARDs such as hydroxychloroquine and sulfasalazine can used in conjunction with Diclofenac. Hydroxychloroquine, an antimalarial drug, is effective in the treatment of rheumatoid arthritis. It is usually used in combination with Diclofenac and sulfasalazine for added benefits. Sulfasalazine is also an effective DMARD. It can reduce symptoms and slow down the joint damage. Leflunomide shows similar effectiveness to Diclofenac and can be used in patients who cannot take Diclofenac(Cuomo Get al., 2006).

IN VITRO -ANTI ARTHRITIC ACTIVITY:

Requirement: Disodium hydrogen phosphate, potassium dihydrogen phosphate, sodium chloride, Egg albumin, diclofenac sodium.

Preparation of buffer solution:

Phosphate buffer saline (6.4)

1. Na_2HPO_4 (Disodium hydrogen phosphate) = 1.18 gm
2. KH_2PO_4 (Potassium dihydrogen phosphate) = 0.9 gm
3. NaCl (sodium chloride) = 8.00 gm
4. Water = 500 ml

Preparation: Dissolve 2.5 g of disodium hydrogen phosphate, 0.19 gm of potassium dihydrogen phosphate, 8.2 gm of sodium chloride in 500 ml of distilled water. Adjust pH of the solution to 6.4 with 1M sodium hydroxide or 1M hydrochloric acid.

Method

Antiarthritic activity diclofenac studied using inhibition of albumin denaturation method. 5 ml of reaction mixture was taken which consist of 0.2 ml of egg albumin, 2.8 ml of phosphate buffer saline (pH – 6.4) and 2 ml of various concentration of diclofenac (20, 40, 60, 80 and 100 ug/ml) were used. Buffer and albumin were served as control. Reaction mixture was incubated at 37°C for min and then at 70°C for 5 min. Reaction mixture was cooled at room temperature and absorbance measured at 660 nm using UV-Visible spectrophotometer (HITACHI). The percentage inhibition of different concentration of ethanol extract of LS and standard drug were calculated by using the following formula,

$$\% \text{ inhibition} = 100 - (A_0 - A_1/A_0) \times 100$$

Where; A_0 was absorbance of control and A_1 was the absorbance of diclofenac.

Statistical analysis:

All data were reported as mean \pm SD triplicates. Data were analyzed by an ANOVA. $P \leq 0.001$ was considered as significant level.

Result and discussion:

The diclofenac sodium significant anti-arthritic activity at 20- 100 $\mu\text{g/ml}$ by protein denaturation inhibition. 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. The maximum activity is exhibited by the

diclofenac at a concentration of 80µg/ml and 100 ug/ml . From the study conducted it can be concluded that diclofenac sodium can be used in the management of arthritis. The results are tabulated and depicted in Table 1.

Table 1.

S. No.	Concentration (ug/ml)	Absorbance	%Inhibition
1	20	0.029	87.91
2	40	0.027	88.75
3	60	0.021	91.25
4	80	0.012	95.00
5	100	0.010	95.83

It is well documented that denaturation of the tissue proteins causes inflammation and followed to the arthritic diseases. The cause of denaturation of protein and amino acids in, in vivo studies have been correlated with the production of auto antigen in certain arthritic disease progressions (Brown JH et al., 1968). The mechanism of denaturation involve alteration in electrostatic, hydrogen bond, hydrophobic and disulphide bonding. From the present study it can be stated that LS is capable of controlling the production of auto-antigen due to In-vivo denaturation of protein in rheumatic diseases (Umopathy et al., 2010).

CONCLUSION

- A study on the diclofenac in vitro was performed. The experiment had shown that diclofenac possess anti-arthritic activity.
- The data regarding model and activity has already been discussed in result. It can be concluded that the anti-arthritic Further studies are required to confirm the exact mechanism underlining the Arthritis and protecting.

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