Research Article

Preclinical evaluation of anti-rheumatoid effect of chrysin in Freund's complete adjuvant induced arthritis in Wistar albino rats

Vishnu R.[1], Krishnan R.[2]

[1]*Assistant Professor, Department of Pharmacology, PK Das Institute of Medical Sciences, Vaniamkulam, Kerala, India*

[2]*Consultant Physiatrist, Indo American Hospital, Brain and Spine Center, Vaikom, Kerala, India*

Received: 1 January 2018 Revised: 30 January 2018 Accepted: 2 February 2018

Abstract

Background: Arthritis is a universal disorder, affecting both sexes and all races. Every human being who lives long enough will have it somewhere, in some degree. Arthritis is one of the leading causes of disability in the world. **Objective:** The present study aimed to find the anti-rheumatoid effect of Chrysin in Freund's complete adjuvant induced arthritis in Wistar albino rats. **Materials and methods:** Thirty rats were divided into 5 groups each of 6 rats. G-I (Normal control), G-II (Freund's complete adjuvant), G-III (Dexamethasone 0.5mg/kg), G-IV (Chrysin 50 mg/kg) and G-V (Chrysin 100 mg/kg). All rats were treated with their respective drugs for 56 days. Blood was collected from each rat for the estimation of serum rheumatoid factor, C-reactive protein, erythrocyte sedimentation rate. **Results:** Rats treated with control showed significant difference with other groups. Dexamethasone significantly reduced the rheumatic parameters. High dose of chrsin groups showed significant difference compared to control and standard drugs. **Conclusion:** From the study observations it can be concluded that administration of Chrysin can significantly reduced the symptoms of rheumatoid arthritis. Further studies required to find the mechanism of action of the test drug.

Keywords: Arthritis, Arthritic index, C-reactive protein, Dexamethasone, Freund's, inflammation

Introduction

Arthritis prevalence as suggested by radiographic surveys rises from 1 % below 30 years of age to over 50 % in people above 60 years of age. Arthritis is one of the leading causes of disability in the world (Tuhina, 2013). The word arthritis is derived from the greek word 'arthro' meaning joint and 'itis' which means inflammation. There are over 100 different forms of arthritis and many causes have been identified (Arya et al., 2013; Parasannasrinivas et al., 2015). The affected individuals seek medical help for joint pain. Moreover the disease affects the patient's work culture resulting in “work absenteeism” and increased frequency of hospital visits (Delisa et al., 2010).

Arthritis is a chronic auto immune disorder that may affect many tissues and organs, skin, blood vessels, heart, lungs and muscles but principally attack the joints, producing a non-suppressive proliferative and inflammatory synovitis. That often progress to destruction of articular cartilage and ankylosis of the joints (Van et al., 1996). Arthritis impairs physical activity, cripples affected individuals and restricts movement to a large extent. In general the arthritis is broadly classified into non-inflammatory and inflammatory arthritis. Osteoarthritis is the commonest non-inflammatory arthritis; it is most usually caused by mechanical joint damage from age or an injury. On the other hand, RA is the commonest inflammatory arthritis. RA is a chronic, progressive, autoimmune, inflammatory disease. It mainly affects synovial joints, producing symmetrical arthritis and is characterized by joint pain and stiffness (Emery et al., 2002). Even though not fatal, patients with RA have reduced life expectancy compared to the general population mainly due to increased prevalence of cardiovascular diseases (Kitas et al 2003). The exact cause for RA remains unknown. However, genetic predisposition, classical CVD risk factors and inflammation related to the disease contribute (Goronzy et al., 1997; Panoulas et al., 2008; Genovese 2005).

The most common form of arthritis is osteoarthritis, also known as degenerative arthritis or degenerative joint disease or osteoarthrosis. It is a group of mechanical abnormalities involving degradation of joints, including articular cartilage and subchondral bone. It is a progressive

*Address for Corresponding Author:
Dr. Vishnu R.
Assistant Professor
Department of Pharmacology
PK Das Institute of Medical Sciences, Vaniamkulam, Kerala, India
Mobile No: +91-9207320999
Email: drvishnuradhakrishnan@gmail.com

disorder of the joints caused by gradual loss of cartilage and resulting in the development of bony spurs and cysts at the margins of the joints (Brandt et al 2009). Other forms of arthritis include rheumatoid arthritis, psoriatic arthritis and related autoimmune diseases. Osteoarthritis is most commonly a disease of the elderly. More than 30% females have some degree of osteoarthritis by the age of 65. About 70% of all osteoarthritis is knee osteoarthritis and the risk for knee increases to 60% in those with a past history of knee injury (Delisa et al 2010). India has the second largest osteoarthritis patient base in the world with over 1.5 crore with women forming a large chunk of this population. Rheumatoid arthritis a progressive auto immune disease. It is the most common systemic inflammatory disease characterized by symmetrical joint involvement. It can occur in any age with increasing prevalence up to seventh decade of life. This disease is three times more common in women. In people aged 15 to 45 years, women predominate by 6:1 ratio, the sex ratio being approximately equal among patients in the first decade of life and in those more than 60 years of age. The main goal of treatment is to relieve joint pain, maintain functions of the joints and to prevent disability. The treatment methods include rest, exercise, physical and occupational therapy, medication, weight control and surgery. The present study aimed to find the anti-rheumatoid effect of Chrysin in Freund's complete adjuvant induced arthritis in Wistar albino rats.

Materials and Methods

Study setting and period

This study was done in the department of Pharmacology, Rajah Muthiah Medical College, Annamalai University in the period of2012-2013.

Animals and grouping

Wister Albino rats weighing 150-200 g were obtained from Central Animal House. They were fed on standard rat pellet diet and water was provided in feeding bottle. All the animals were maintained under standard laboratory conditions temp 24°C and humidity 60-70%. The study was approved by Institutional Animal Ethics Committee (Registration No. 160/1999 CPCSEA).

Group-I: Normal control (Distilled water)

Group-II: Freund's complete adjuvant

Group-III: Dexamethasone 0.5 mg/kg

Group-IV: Chrysin 50 mg/kg

Group-V: Chrysin 100 mg/kg

Anti-rheumatoid study

Experimental arthritis was induced by injecting 0.1 ml of 0.5% of Freund's complete adjuvant into the tibio tarsal joint of the left hind paw intra-articularly in group II to V. Drug treatment was started with 0.5 mg/kg of dexamethasone in group III, Chrysin 50 mg/kg in group IV and Chrysin 100 mg/kg in group V from the first day and continued till 56th day. The animals were observed for primary lesions on 1-7 days. Secondary lesions involving other non-injected sites which may appear after 10th day and were graded by a scoring method known as Schorlemmes method. (Silman et al., 1993). Blood samples were collected for Erythrocyte Sedimentation Rate, C - reactive protein, and Rheumatoid factor estimation by intraocular puncture on days 1, 7, 14, 28 and 56 (Snekhalatha et al., 2013).

Statistical analysis

The data was analyzed by Statistical Package for Social Sciences (Version 16.0). One ANOVA (Post hoc) followed by Dunnet t test applied to find the statistical significant between the groups. P value less than 0.05 ($p<0.05$) considered statistically significant at 95% confidence interval. The data was expressed in mean and standard deviation.

Results

Rats treated with FCA showed significant increase in ESR compared to control group in all the days. Co-administration of dexa significantly reduced the ESR count compared to group-II. High dose of Chrysin administration significantly prevented the FCA induced increase in ESR count. Maximum effect was seen with high dose of Chrysin then low dose groups. Test drug showed significant difference compared to dexa group (Table 1). Significant

Table 1. Effect of Chrysin on Erythrocyte Sedimentation Rate in Freund's complete adjuvant (FCA) induced arthritis

Groups	ESR (DAY 1) mm/hour (MEAN ± SD)	ESR (DAY 7) mm/hour (MEAN ± SD)	ESR (DAY 14) mm/hour (MEAN ± SD)	ESR (DAY 28) mm/hour (MEAN ± SD)	ESR (DAY 56) mm/hour (MEAN ± SD)
G-I	9.667±1.211^{a}	8.500±1.049^{a}	8.333±2.422^{a}	7.166±1.472^{a}	7.500±1.049^{a}
G-II	31.000±1.414^{d}	39.667±1.366^{d}	42.667±1.752^{e}	36.000±1.789^{d}	30.167±1.472^{d}
G-III	11.166±1.472^{b}	11.833±1.472^{b}	13.667±1.032^{b}	19.000±2.367^{b}	19.166±1.472^{b}
G-IV	18.000±0.894^{c}	31.333±1.633^{c}	30.000±1.414^{d}	28.833±1.169^{c}	27.667±1.751^{c}
G-V	17.500±1.225^{c}	30.500±1.871^{c}	25.000±5.478^{c}	21.000±2.367^{b}	19.500±1.643^{b}

(Values not sharing a common superscript differ significantly at $p<0.05$)

Table 2. Effect of Chrysin on C-reactive protein in freund's complete adjuvant (FCA) induced arthritis

Groups	CRP (DAY 1) mg/dl (MEAN±SD)	CRP (DAY 7) mg/dl (MEAN±SD)	CRP (DAY 14) mg/dl (MEAN±SD)	CRP (DAY 28) mg/dl (MEAN±SD)	CRP (DAY 56) mg/dl (MEAN±SD)
G-I	6.833±0.916^{a}	6.550±0.999^{a}	6.000±0.767^{a}	6.400±0.885^{a}	6.233±0.914^{a}
G-II	18.667±2.805^{c}	21.067±2.405^{c}	20.600±2.039^{d}	24.900±1.263^{d}	24.100±0.745^{d}
G-III	9.200±1.226^{b}	12.417±1.189^{b}	10.550±2.170^{b}	8.450±1.449^{b}	7.617±1.211^{a,b}
G-IV	9.367±1.777^{b}	12.300±1.653^{b}	12.650±0.933^{c}	10.133±1.464^{c}	10.167±1.950^{c}
G-V	8.633±1.417^{a,b}	12.050±1.509^{b}	12.367±0.786^{c}	8.700±1.192^{b,c}	8.283±1.121^{b}

(Values not sharing a common superscript differ significantly at $p<0.05$)

Table 3. Effect of Chrysin on rheumatoid factor in freund's complete adjuvant (FCA) induced arthritis

Groups	RAF (DAY 1) IU/dl (MEAN±SD)	RAF (DAY 7) IU/dl (MEAN±SD)	RAF (DAY 14) IU/dl (MEAN±SD)	RAF (DAY 28) IU/dl (MEAN±SD)	RAF (DAY 56) IU/dl (MEAN±SD)
G-I	16.400±1.339^{a}	17.167±1.411^{a}	16.733±1.204^{a}	16.033±1.461^{a}	15.433±1.267^{a}
G-II	157.333±14.841^{d}	187.833±12.529^{c}	194.333±6.861^{c}	205.833±7.026^{d}	193.333±7.448^{b}
G-III	125.000±6.324^{b}	154.333±16.071^{b}	59.000±10.178^{b}	25.667±8.140^{b}	15.400±3.356^{a}
G-IV	137.667±8.334^{c}	166.167±14.456^{b}	64.167±8.818^{b}	42.333±7.554^{c}	18.500±5.822^{a}
G-V	132.667±10.764^{b,c}	164.000±12.837^{b}	57.500±9.268^{b}	26.667±10.801^{b}	16.400±1.447^{a}

(Values not sharing a common superscript differ significantly at $p<0.05$)

increase in C-reactive protein levels was observed in day 1, 7th, 14th, 28th and 56th day compared to control group. G-III, IV and V showed significant reduction in C-reactive protein levels compared to G-II in all the days (Table 2). Day 1st, 7th, 14th, 28th and 56th day showed increased rheumatoid factor levels in group-II compared group-I it was statistically significant. Group-III, IV and V showed reduced rheumatoid factor levels compared to G-II. G-V showed more effect than G-IV (Table 3).

Discussion

Arthritis is a chronic auto immune disorder that impairs physical activity and restricts movement to a large extent. Arthritis may be non-inflammatory or inflammatory arthritis. Rheumatoid Arthritis is the commonest form of inflammatory arthritis. The incidence of rheumatoid arthritis is 0.75 percent in India which necessitates the discovery of newer drugs with greater pharmacological actions and minimal side effects as novel, approach to treatment modalities, which includes rest, exercise, weight control and surgery.

Chrysin also known as “Blue passion flower” originating from south America. It is a dihydroxy flavone. It is found to have antitumour properties in '*in-vitro'* cells of oesophageal and colon cancer cell lines (Boon et al., 2010). It is also found to have anti-diabetic properties (Lukacinova et al., 2008). As a flavonoid it is reported to be a good anti-oxidant (Teng et al., 2005). *In vitro* studies of oral administration have shown that Chrysin has anti inflammatory action which prompted us to undertake this study, where in we have evaluated, the anti arthritic effect of Chrysin in Freunds complete adjuvant induced arthritis.

We also compared its efficacy with Dexamethasone, one of the conventionally used drugs in the treatment of arthritis. ESR is a non specific measure of inflammation. During the process of inflammation a high proportion of fibrinogen in blood, causes RBC's to stick to each other. ESR is the rate at which red cells sediment in a period of one hour. ESR was introduced by Westergrens in 1921. In our study, FCA has increased the ESR considerably, from day one onwards. With the peak effect on day 7 and afterwards has shown a slight reduction on days 28 and 56. The reason being, ESR increases to a greater extent with moderate increase in fibrinogen, an acute phase protein (Tarik et al., 2002). It is an established fact that about 60-70 percent of an increase in ESR is due to fibrinogen as ESR is an indirect measure of acute phase reaction.

Dexamethasone being an effective anti-inflammatory agent has reduced the FCA induced increase in ESR considerably on all days. The action of Chrysin at 100 mg on days 14, 28 and 56 are comparable to dexamethasone group. This indicates Chrysin can be more effective on chronic arthritis. The significance of ESR values in chronic rheumatoid arthritis has been reported by (Wolfe et al., 1994). However ESR is also sensitive to various factors like gender, age, pregnancy, drugs like salicylate, smoking, and level of plasma proteins, storage temperature, and method of assay. All these factors do not affect the C reactive protein (CRP) value and hence CRP is a better marker in acute phase. The inhibitory action of Chrysin on ESR indicates that it has an effect on fibrinogen which is responsible for

acute phase of inflammation.

C reactive protein discovered in 1930 by Tillet and Francis, binds to phosphocholine binding sites of foreign pathogens and damaged host cells (Volkanis 14th Ed). CRP concentration increases during the acute phase, usually within 4 hours and peaks within 24-72 hours. When the underlying inflammation resolves, it returns back to normal value. CRP values can accurately reflect the level of inflammation or tissue injury in Rheumatoid arthritis. The local reactions like vasodilatation and platelet aggregation, neutrophil chemotaxis and systemic reaction like fever, leukocytosis is termed as "acute phase reactions". The acute phase proteins like CRP and ESR will help not only in diagnosis of inflammation but also to find out about the efficacy of the drugs given. CRP is consistently, increased during the acute phase (Ist week) in the FCA group. The FCA induced increase in CRP was found to be reduced drastically by Dexamethasone treated group during the same period. Our drug Chrysin at 50 mg was equi-effective to Dexamethasone on day 1 and more effective than even dexamethasone at 100mg dose on day 1 proving that it is a good anti-inflammatory agent and can be employed in the treatment of Rheumatoid arthritis. Since CRP level is not a specific marker for chronic inflammation, although the anti-inflammatory effect of Chrysin at 50 mg dose levels is significant as per our study on days 14, 28 and 56 and comparable to the action of Dexamethasone, it is too early to say if it will be effective in chronic inflammation, but still can be claimed to be useful in rheumatoid arthritis. However further clinical studies are needed to prove the usefulness of chrysin in rheumatoid arthritis.

Although the exact role of Rheumatoid factor in pathogenesis of rheumatoid arthritis has not been well established, these antiglobulin antibodies are found in 75-80% of rheumatoid patients (Stephen et al., 2010). Rheumatoid factor is one of the criteria proposed by American college of Rheumatology for diagnosis of Rheumatoid arthritis (Mottonen, 1998). FCA has increased the level of Rheumatoid factor upto 28 days and after that on day 56, the value has reduced slightly. Dexamethasone has reduced FCA induced increase in Rheumatic factor drastically and its effect is greater from day 14 onwards. It has resulted in 70% inhibition on day 14, 88% on day 28 and 93% on day 56 (near normal). Our drug Chrysin at 50mg dose resulted in 68% inhibition of RAF on Day 14, 71 % inhibition on day 28 and 91% inhibition on day 56. Similarly Chrysin at 100mg dose resulted in 71% inhibition on Day 14, 88% inhibition on day 28 and 92% inhibition on day 56. At both 50 and 100mg, Chrysin, was equi effective to Dexamethasone and its effect was found to be on par with control values. At 100mg dose, on all three days its efficacy was found to be similar to dexamethasone.

Although rheumatoid factor is not a specific indicator for rheumatoid arthritis its presence is of prognostic significance because high values indicate the severity of the condition. In 5% of healthy population rheumatoid factor is present and it increases as the age advances. However in chronic polyarthritis, high titre IgM rheumatoid factor is specific for the diagnosis rheumatoid arthritis. In our study the drug Chrysin was very effective in bringing down the rheumatoid factor level and hence can be claimed useful in the treatment of rheumatoid arthritis. This study results showed that Chrysin is useful in the treatment of rheumatoid arthritis and other immune disorders.

Conclusion

Administration of Chrysin reduced the symptoms of arthritis. It can be used in the treatment of rheumatoid arthritis and other inflammatory joint disorders. Further studies required to find out the mechanism of action, adverse effects of the drugs.

Conflict of interest: Nil

References

Ajay DK, Prashant VP, Uday NH, Rabindra KN, Haidarali MS. 2014. Anti-inflammatory and artharthritic activity of anthraquinone derivatives in rodents. International Journal of Inflammation, 2(1):1-12.

Arya RK, Jain V. 2013. Osteoarthritis of the Knee Joint - An Overview. Journal of Indian Academy of Clinical Medicine, 14(2):154-162.

Ashman RB, Papadimitriou JM. 1995. Production and function of cytokines. Immunology & Cell Biology, 18(1):23-30.

Boon YK, Siang Lc, Prabha B. 2010. Apoptotic effects of chrysin in Human Cancer Cell Lines. International Journal of Molecular Science, 11:2188-2199.

Brandt Kenneth D, Dieppe P Radin Eric. 2009. Etiopathogenesis of osteoarthritis. Medical Clinics of North America, 93(1):1-24.

Delisa AJ, Walter RF, Bruce MG, Nicolas EW, Lawrence RR. 2010. Rehabilitation of the patient with Rheumatic diseases. Lippincott Williams & Wilkins, 40:1015-1021.

Delisa AJ, Walter RF, Bruce MG, Nicolas EW, Lawrence RR. 2010. Rehabilitation of the patient with rheumatic diseases. Lippincott Williams & Wilkins, 40:1015-1021.

Emery P, Foster W and Suarez M. 2002. Rheumatoid arthritis. Clinical Evidence, 5:1107-1121.

Genovese MC. 2005. Abatacept for Rheumatoid Arthritis Refractory to TNF-α inhibition. New England Journal of Medicine, 353:1114-1116.

Goronzy JJ, Weyand CM. 1997. Rheumatoid arthritis:

Epidemiology, Pathology and pathogenesis. primer on the rheumatic diseases. Arthritis Foundation, 2:155-162.

Kitas GD, Erb N. 2003. Tackling Ischemic Heart Disease in Rheumatoid Arthritis. Rheumatology, 42:607-613.

Lukacinova A, Moizis J, Benacka R, Keller J, Maguth T, Kurila P. 2008. Preventive effects of flavonoids on Alloxan-induced Diabetes Mellitus in Rats. Acta Veterinaria Brno, 77:175-182.

Mottonen TT. 1998. Prediction of erosiveness and rate of development of new erosions in early rheumatoid arthritis. Annals of the Rheumatoid Diseases, 47:648-653.

Panoulas VF, Metsios GS, Pace AV, John H. 2008. Hypertension in Rheumatoid Arthritis. Rheumatology, 47:128612-98.

Parasannasrinivas D, Karthikeya P, Mahima V, Guledgud, Reema SD. 2015. Diagnostic imaging in TMJ osteoarthritis: A case report and overview. International Journal of Dental Science Research, 3(3):56-59.

Snekhalatha U, Anburajan M, Venkatraman B, Menaka M. 2013. Evalaution of complete freund's adjuvant induced arthritis in a Wistar rat model. Comparison of thermography and histopathology. Journal of Rheumatology, 72(4):375-382.

Stephen JM, Gary DH. 2010. Pathophysiology of Rheumatoid disease, 6th Edition., pp 667.

Tarik MH, David HK. 2002. C-reactive protein and Erythrocyte Sedimentation Rate in Orthopaedics. The University of Pennsylvania Orthopedic Journal, 15:13-16.

Teng GG, Turkiewicz AM, Moreland LW. 2005. Abatacept: A costimulatory inhibitor for treatment of rheumatoid arthritis. Expert Opinion Inbiologicals Therapy, 5:1245.

Tuhina N. 2013. The epidemiology and impact of pain in osteoarthritis. Oseoarthritis Cartillage, 21(9):1145-1153.

Van der heide A, Jacobs JW. 1996. The effectiveness of early treatment with second line anti – rheumatic drugs. Annals of Internal Medicine, 124:699-707.

Volkanis JE. 2000. Acute Phase Proteins in Rheumatic disease. Arthritis and allied conditions. 14th ed., pp 504-14.

Wolfe F, Mitchell DM, Sibley JT, Fries JF, Bloch DA, Cathy MA. 1994. Mortality of Rheumatoid arthritis. Arthritis and Rheumatism, 37(4):481-494.