ISSN: 0976-3031

Available Online at http://www.recentscientific.com

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 8, Issue, 10, pp. 21020-21024, October, 2017

International Journal of Recent Scientific Research

DOI: 10.24327/IJRSR

Research Article

EVALUATION OF ANTIARTHRITIC POTENTIAL OF *CURCUMA CAESIA ROXB* RHIZOME

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DOI: http://dx.doi.org/10.24327/ijrsr.2017.0810.1000

ARTICLE INFO

Article History:

Received 10th July, 2017
Received in revised form 14th August, 2017
Accepted 08th September, 2017
Published online 28th October, 2017

Key Words:

Anti-arthritic, *Curcuma caesia Roxb,* Autoimmune disease, Freund's Complete Adjuvant (FCA), Indomethacin.

ABSTRACT

The objective of present study was to investigate the anti-arthritic effect of ethanolic and aqueous extract of *Curcuma caesia Roxb.* rhizomes on Freund's Complete Adjuvant (FCA) induced arthritis in male Wister rats. Rheumatoid arthritis (RA) is a chronic, painful autoimmune inflammatory disease which affects people especially women in high rate. The rhizomes of *Curcuma caesia Roxb.* were collected, shade dried and the dried material was extracted with ethanol by maceration process. The aqueous extract was obtained by maceration with distilled water. The rats were divided into 4 groups comprising of 6 animals in each group as Control Group, Standard Group (Indomethacin 10mg/kg), Test Group-I, 200mg/kg.p.o. and Test Group-II, 200 mg/kg.p.o**.** for anti-arthritic activity. Following parameters have been selected to evaluate the anti-arthritic effect such as paw volume, body weight, SGOT, SGPT, and hematological profile WBC, RBC, ESR and hemoglobin content were observed. The results indicate that paw edema is reduced in all treated groups compared with control group. The significant recovery were observed in body weight and hematological profile compared with control group. The level of SGOT and SGPT were not changes in drug treated groups except standard group. The data showed 200mg/kg ethanolic extract is highly potent as compared to aqueous extract. The ethanolic extract of *C. caesia Roxb* may be beneficial for arthritis.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease that affects nearly1% of the world's adults (Kahlenberg and Fox, 2011). RA affecting more than 1.3 million Americans of these, about 75% is women (Gravallese *et al*., 1998). In fact, 1–3% of women may get rheumatoid arthritis in their lifetime. RA mostly begins between the fourth and sixth decades of life. However, it can start at any age. Patients with arthritis experience swelling in the joints, synovial tissue inflammation and subsequent damage to the cartilage which result in significant disability and decrease in the quality of life (Ackerman *et al*., 2017). In rheumatoid arthritis body's immune system starts to attack its own body tissue. The most damage occurs to the joints lining and cartilage which eventually result in erosion of two opposing bones. Inflammation in joints results in pain and stiffness which lead to progressive joint damage resulting in deformities and loss of function. Rheumatoid arthritis often affects joints in the fingers such as wrists, knees and elbows. In children, the disorder can present with a skin rash, fever, pain, disability and limitation in daily activities (Brooks, 2006). The immune system contains a complex organization of cells and antibodies designed normally to "seek and destroy" invaders of the body, particularly infections. Patients with autoimmune diseases have antibodies in their blood that target their own body tissues, where they can be related with inflammation (Lindqvist *et al*., 2006). Because it can affect multiple other organs of the body, rheumatoid arthritis is referred to as a systemic illness and is sometimes called rheumatoid disease. The rheumatoid arthritis is mediated by an inter-related network of cytokines, proteolytic enzymes and prostanoids. IL-1, TNF-alpha, etc. are pro-inflammatory cytokines and are the central mediators in the disease. Raised levels of IL-1 can also be seen in synovial fluid of some patients as primary cell mediated response in Rheumatoid arthritis (Ruderman and Tambar, 2012). Treatment options depending on the type of rheumatoid arthritis and treatment include physical therapy, lifestyle changes (including exercise and weight control), bracing, and medications. Joint replacement surgery may be required if the medication does not reduce the inflammation. Medications can helpful to reduce inflammation. Regularly prescribed medications for rheumatoid arthritis are non-steroidal anti-inflammatory agents (NSAIDs), corticosteroids, and disease modifying anti-rheumatic drugs (DMARDs) (Ideguchi *et al*., 2006). However, these drugs having higher side effects. The herbal drugs now a day used for treatment of arthritis. The extracts of *Curcuma caesia Roxb.* rhizomes were selected for the anti-arthritic activity (Baghel *et*

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al., 2013). The plant having vital bioactive constituents such as ar-turmerone, (Z)-ocimene, ar-curcumene, 1, 8-cineole, elemene, borneol, bornyl acetate, camphor and curcumene (Pandey *et al*., 2003). These active constituents are helpful for the treatment of arthritis. *Curcuma caesia Roxb.* rhizomes in arthritic rats were tested rigorously by scientifically controlled experiment. This study has been used extensively to analyse the anti-arthritic effect of rhizome extract of *Curcuma caesia Roxb.* in FCA induced arthritis in Wistar albino rats.

MATERIALS AND METHODS

Materials

Instruments

Plethysmometer, microtome, hemoglobinometer, auto-analyser, ESR stand, microcope, soxlet apparatus, lypholyser and vaccum evaporator.

Drugs and Chemicals

Freund's complete adjuvant (FCA), Indomethacine, SGOT and SGPT commercial kit.

Methods

Collection of plant Rhizomes

The rhizomes of *Curcuma caesia Roxb* was collected from Bhopal (M.P) India during October- November. It was made completely clean and dust free.

Authentication of Plant

The plant material was identified and authetified by Prof. P. Patil (Prof & HOD) Department of botany, Government M.L.B Girls Autonomous College Bhopal (M.P) and specimen voucher no.308.

Drying and Size Reduction of Plant Material

The rhizomes were dried under shade for 6-7 days. It was pulverized to coarse powder with the help of hand grinder. The coarse powder was packed in to airtight container and stored in cool and dry place. The course powder was used for the further study.

Preparation of Crude Extract

Ethanolic extract

The powdered rhizomes (250g) were extracted with ethanol (70:30) solvent by maceration process for about 7 days. Solvent was concentrated under reduced pressure using rotatory evaporator and dried below the temperature 40^0C. The extract was brown in color and the percentage yield of the extract was calculated (Gupta *et al*., 2014).

Aqueous extract

The powdered Rhizomes (250g) were macerated with distilled water for 7 days. Ethanol (2ml) was added to prevent the growth of microorganism in the extract. Solvent was concentrated under reduced pressure using rotatory evaporator and dried below the temperature 40^0C (Handa *et al*., 2008). The extract was brown in colour and the percentage yield of the extract was calculated.

Qualitative phytochemical analysis

The crude extract obtained by solvent extraction was subjected to various qualitative tests to detect the presence of common chemical constituents as: Alkaloids, Glycosides, Carbohydrates, Flavonoides, Tannins and phenolic compounds. (Handa *et al*., 2008)

Animal care and handling

Male albino Wister rats weighing 150-250g were used in experiment. The experimental animals were maintained under standard laboratory condition in an animal house. The whole research work was carried out under the regulation of CPCSEA. All animals were kept in animal house, SIRT-Pharmacy. The research work was performed in Pharmacological laboratory at SIRT-Pharmacy Bhopal, MP.

Acute toxicity studies

This study is needful before pharmacological screening on animals. The acute oral toxicity study was carried out according to OECD 425 guideline (Organization for Economic Coorperation and Development) which is based on a stepwise procedure with the use of a minimum number of animals per step. The ethanolic and aqueous extracts of *curcuma caesia Roxb* 2000mg/kg were administered to Wister albino rats. The sign and symptoms of acute toxicity were examined for the effect of extract on general behavior for 48 hours and further upto 14 days for delayed toxicity (Rispin *et al*., 2002).

Anti-arthritic activity

Freund's complete adjuvant induced arthritis in rats

In the experiment rats was divided into 4 groups comprising of 6 animals in each group as follow:

Control Group: Treatment with control saline (5ml/kg.p.o) + FCA
Standard Group: 10mg/kg i.p Indomethacin + FCA

Test Group-I 200mg/kg ethanolic extract of *C. caesia Roxb.* + FCA
Test Group-II: 200mg/kg aqueous extract of *C. caesia Roxb.*+ FCA

Freund's Complete Adjuvant (FCA) arthritis rats

In Freund's Complete Adjuvant (FCA) arthritis model, 0.5 mL of FCA was injected into the left hind knee joint. The animals were divided into the four groups (n = 6). FCA was injected to all animals. The animals were anesthetized by using ketamine and volumes of the hind paw and knee joint were measured by mercury Plethysmometer at 0, 7, 14, 21 and 28 days after oral administration (Kim *et al*.,2016).

Parameters

Paw Volume

The severity of adjuvant arthritis was quantified by measuring the volume of the hind paw using Plethysmometer. Paw volume (ml) was measured at 0 day and thereafter 7, 14, 21 and 28 days of FCA injection. (DeCastro *et al*., 1981).

Body weight

The body weight was measured on 0 day and thereafter 7, 14, 21 and 28 days of FCA injection to each rat by using single pan digital balance.

Biochemical study

The Biochemical estimation was carried out on the last day of the study. This study was applied on group of Wister albino rats. Blood samples were collected from retro-orbital venous plexus of rats, after 12 hours. Two millimeters of blood were collected in a clear graduated centrifugation tube, left to clot at room temperature in a water bath for 15 minutes, and then centrifuged at 3000 rpm (rotation per minute). The supernatant serum was collected in a dry clean tube. Serum was separated by centrifugation at 2500 rpm at 30°C for 15 min. Serum samples were immediately subjected to biochemical estimation of serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) for liver toxicity (Ramachandra *et al*., 2007).

Hematological study

Blood samples were collected from the retro orbital plexus of rat for laboratory tests on day 29th after FCA injection. The hematological parameters: Red Blood Cell (RBC), White Blood Cell (WBC) was estimated by Haemocytometer, Erythrocyte Sedimentation Rate (ESR, mm/h) by Wintrobe tube method and Hemoglobin content (Hb) were estimated by Sahil heliage Haemoglobinometer (Kappor *et al*., 2009).

Histopathology Study

Histopathology samples from hind paw were taken at 29th day and microscopic slides were prepared using haematoxylin and eosin staining (Bendele *et al*., 1999). The paws were ampulated above the knee joint and were fixed in 70% formaldehyde solution. The paws were then decalcified, embedded in paraffin and sectioned in a mid-sagittal plane. Uninflammed decalcified joint at the same time were used as a control sample. The sample were embedded in paraffin and sectioned with microtone (5um). Then microscopic slides were examined fewer than 40 X lens of projected microscope (Ekambaram *et al*., 2010).

RESULTS

Percentage yield: The percentage yield of alcoholic and aqueous extract of *C. caesia Roxb.* rhizome was calculated and found 4.70% and 3.69% respectively.

Preliminary phytochemical analysis: The qualitative phytochemical analysis indicate the presence of active constituents in ethanolic and aqueous extracts such as carbohydrates, alkaloids, glycosides, phenolic compound, saponin, flavonide, tannin, and steroids.

Acute oral toxicity study: Acute oral toxicity study was conducted as per OECD guideline 425. At higher dose 2000mg/kg p.o. has been selected for acute toxicity study. There were no sign and symptoms observed from 4 hours to 14 days of toxicity study in ethanolic and aqueous extracts.

FCA induced paw edema in rats: In the present study the control group showed significant *(P<0.001)* increase in paw edema from 7 day to 28 day. Standard and test group- I showed significant *(P<0.01)* reduction in paw edema on 14 day to 28 day. However ethanolic extracts 200mg/kg showed significant reduction on 7 day to 28 day when compared with control group.

Body weight: The body weight is subsequently decline 14 day to 28 day in control group. Standard and test group-II showed the improvement in body weight of arthritic rats. However test group-I, could not improve the body weight when compared with control group.

Hematological profile: In the present study decrease hemoglobin content in blood has been observed on 29 day of study in control group. However significant *(p≤ 0.01)* improvement in drug treated group. The ESR level was increased in control group. The standard and test group-II were improved the ESR level. However test group-I did not showed significant improvement in ESR level compared with control group. The RBC content was decreased in control group but drug treated groups showed significant recovery compared with control group. WBC count was decreased in control group however, significant *(p≤0.001)* maintained WBC count in standard, test group-I and test group-II.

Biochemical parameters: In the present study biochemical parameters were observed on 29 day. The control group showed non-significant change in SGOT and SGPT level. However the standard group shows significant change in SGOT and SGPT level compare with initial value. Extract treated test group-I and II showed non-significant change in SGOT and SGPT level.

Histopathology of Arthritic left knee joint of rat

The histopathological study indicates that the destruction of joint in control group, however in drug treated groups recovery was observed. The results of the microscopic examinations were shown below in figures.

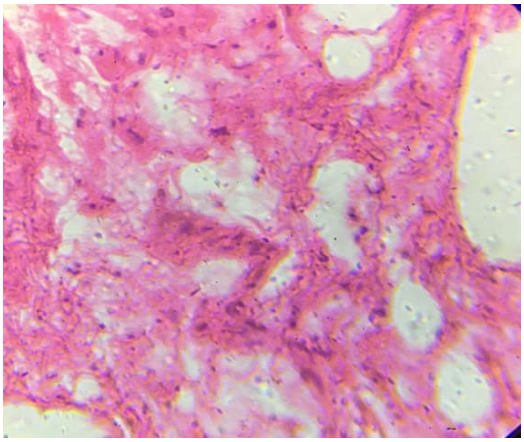

Fig 1 Group-1 Arthritic Control (FCA treated+ vehicle treated)

Photomicrograph of knee joint from adjuvant arthritic rat 28 days post adjuvant injection at the sub-planter region of left hind paw. Inflammation with marked edema is present with bone destruction and synovial membrane depletion was observed.

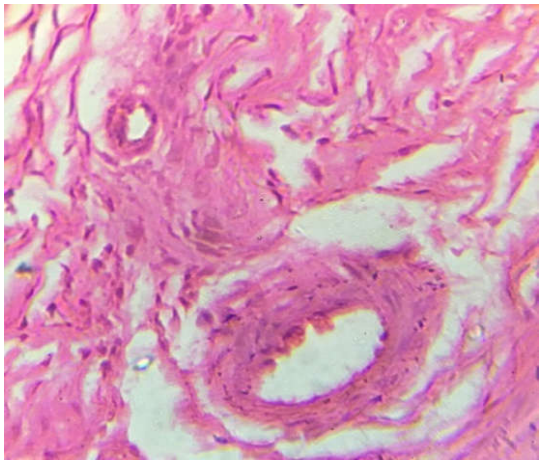

Fig 2 Group-2 Standard group Photomicrograph of knee joint from adjuvant arthritic rat 28 days post adjuvant injection at the sub-planter region of left hind paw. The mild inflammation with nil edema around the bone, bone destruction and synovial membrane depletion was negligible.

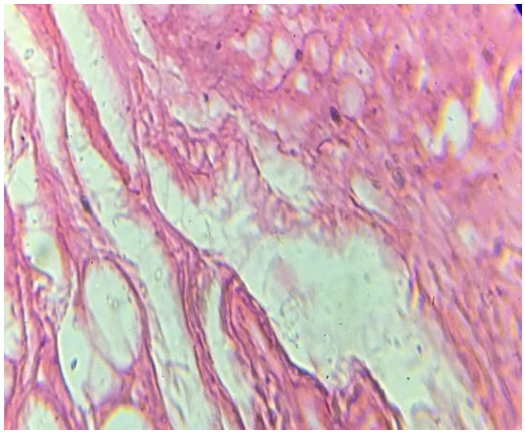

Fig 3 Group-3 Test group-I (FCA treated + Ethanolic extract 200mg/kg treated) Photomicrograph of knee joint from adjuvant arthritic rat 28 days post adjuvant injection at the sub-planter region of left hind paw. The mild inflammation with nil edema around the bone, with minor bone destruction and synovial membrane depletion was observed.

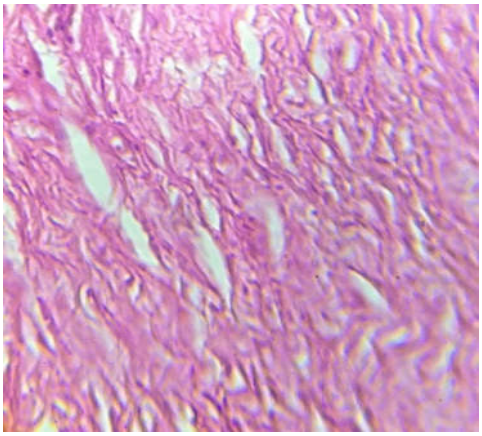

Fig 4 Group-4 Test group-II (FCA treated + aqueous extract 200mg/kg treated) photomicrograph of knee joint from adjuvant arthritic rat 28 days post adjuvant injection at the sub-planter region of left hind paw. The mild inflammation with nil edema around the bone, bone destruction was not seen and minor synovial membrane depletion was observed.

Anti-Arthritic Activitv

Table 1 Effect of *C. caesia Roxb.* rhizome extracts on FCA induced paw edema in rats

Groups Treatment	Dose (mg/kg)	Paw volume (ml)				
		0 day	7 day	14 day	21 day	28 day
Control Group	5ml/kg saline + 0.5ml FCA	0.01±0.02	2.325±0.154	2.344±0.163***	2.57±0.133***	2.587±0.158**
Standard group	10 mg/kg	0.01±0.02	2.615±0.066	1.406±0.338*	1.03±0.25**	0.76±0.239**
Test group-I	200 mg/kg Ethanolic Extract	0.035±0.04	1.207±0.23**	0.9±0.293**	0.6±0.248***	0.47±0.27***
Test group-II	200mg/kg Aqueous extract	0.01±0.01	1.869±0.283	1.575±0.154***	1.465±0.066*	0.82 ±0.08***

Each value is expressed as mean ± SEM (N=06), the level of significance in paw edema were analyse by dennets multiple range comparision test. All groups compared with 0 day, P value less then *P<0.05, **P<0.01, ***P<0.001 considered as significant.

Table 2 Effect of *C. caesia Roxb.* rhizome extracts change in body weight of arthritic rats

Groups Treatment	Dose (mg/kg)	Body weight (gm)				
		0 day	7 day	14 day	21 day	28 day
Control Group	5ml/kg saline 0.5ml FCA	232.3±21.07	232.3±21.07	207.4±15.73*	191.8±16.82*	155.4±8.6**
Standard group	10mg/kg	265±32.45	232.4±37.02	248.4±26.14	241.7±26.2*	242.8±26.1*
Test group-I	200mg/kg Ethanolic Extract	268.4±25	259±25	246±20.51	256±20.51	265±21.0*
Test group-II	200mg/kg Aqueous extract	200±18.29	193±16.23	200±18.29	192.7±16.73	192.7±16.73

The values are expressed as mean ± SEM (N=06), Control group compared with normal group and drug treated group compared with a control group P value less *P<0.05, * * P<0.01, ***P<0.001 considered as significant

Table 3 Effect of *C. caesia Roxb.* rhizome extracts on hematological parameters

Groups Treatment	Dose Mg/kg	Hb%	ESR(mm)	RBC (million/cmm)	WBC
Control group	5ml/kg saline +0.5ml FCA	7.4±0.7	34.4±5.6	3.3±0.12	12±0.23
Standard group	10mg/kg	13.4±1.02**	16.74±6.03**	6.3±0.11***	11±0.22***
Test group-I	200mg/kg Ethanolic Extract	12.3±1.12**	18.2±9.93*	5.5±0.12***	11±0.14***
Test group-II	200mg/kg Aqueous extract	11.3±0.8**	24.63±7.11	4.2±0.22**	11.5±0.13***

The values are expressed as mean ± SEM (N=06), Control group compared with drug treated group, P value less then *P<0.05, ** P<0.01, ***P<0.001 considered as significant.

Table 4 Effect of *C. caesia Roxb.* rhizome extracts on biochemical parameters

Groups Treatment	Dose mg/kg	SGOT	SGPT
Control group	5ml/kg saline 0.5ml FCA	150.8±0.89	68.11±0.47
Standard group	10mg/kg	139.08±0.67***	58.41±0.72***
Test group-I	200mg/kg Ethanolic extract	142.02±4.03	64.2±0.31
Test group-II	200mg/kg. Aqueous extract	144.69±1.57	66.13±0.82

The values are expressed as mean ± SEM (N=06), Control group compared with drug treated group, P value less then* P<0.05, ** P<0.01, ***P<0.001 considered as significant.

DISCUSSION

In the present study Freund's complete adjuvant was used to induce arthritis in rats. To investigate the potential effect of ethanolic and aqueous extracts of *C. caesia Roxb.* rhizome in arthritic rats. Acute toxicity study showed the non toxic nature of both extracts of *C. caesia Roxb.* There was no lethality or any toxic reaction found with selective dose at the end of study

period. The dose of test drug has been selected on the basis of standard guidelines. Several active constituents of *C. caesia Roxb*. rhizome extracts has been reported to possess anti-inflammatory property. It has been reported that ar-turmerone, (Z)-ocimene, ar-curcumene, 1, 8-cineole, elemene, borneol, bornyl acetate, camphor and curcumene are the major active constituents having anti-inflammatory activity. These active constituents reduce the production of prostaglandins and also suppress the TNF induced NF-kB. Especially curcumene modulates the anti-inflammatory response by down regulating the activity of Cycloxygenase-II (COX-II). Lipoxygenase and Inducible nitric oxide synthase (iNOS) enzymes inhibit the production of cytokines. The steroids block the allergic reactions and reduce the symptoms such as itching, swelling and redness of skin. In the present study, the control group showed increase paw edema on 14th day to 28th day and drug treated group showed the significant reduction in paw edema on 14th day to 28th day. 200mg/kg ethanolic extract showed effective results to reduce the paw edema. The body weight decline 14th day onwards. However, the drug treated groups showed improvement in body weight. The hematological parameters altered in control group but in the drug treated groups recovery was observed. The liver functions were also elevated. There were no changes in groups except standard group. The histological study of knee joint of arthritic rat showed destruction of joint in control group. However, in the drug treated groups the recovery was observed. Ethanolic extract of *C. caesia Roxb*. rhizome showed highly protective effect.

CONCLUSION

In conclusion it can be stated that the ethanolic and aqueous extracts of *C. caesia Roxb.* rhizome has a beneficial effect in long lasting reduction in rats paw edema and recovery in hematological changes. It is also showed protective effect on arthritic rat joints which is mediated by inhibition of prostaglandin synthesis as well as central inhibitory mechanisms. The histological study indicates that the plant extracts showed protective effect. The ethanolic extract is highly effective as compared to aqueous extract. The ethanolic extract may be beneficial for the treatment of arthritis for future aspects.

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