Journal of Applied Pharmaceutical Science Vol. 5 (08), pp. 046-053, August, 2015
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2015.50808
ISSN 2231-3354 

Evaluation of in-vivo antiarthritic potential of methanolic extract of *Costus speciosus* rhizome

Chandra Kala[1], Syed Salman Ali[2*], Mohd. Abid[2], U S Sharma[3], Najam Ali Khan[2]

[1]Department of Pharmaceutical Technology, MIET, N.H. 58, Delhi-Roorkee Highway, Baghpat Road Bypass Crossing,Meerut-250005, UP, India.
[2]School of Pharmaceutical Sciences, IFTM University, Lodhipur rajput, Delhi road (NH-24), Moradabad – 244102, Uttar Pradesh, India.
[3]Sir Madanlal Institute of Pharmacy, Alampur Hauz, Agra Road, Etawah -206001 (UP), India.

ARTICLE INFO

Article history:
Received on: 24/04/2015
Revised on: 16/05/2015
Accepted on: 19/06/2015
Available online: 28/08/2015

Key words:
Costus speciosus, Freund's complete adjuvant, Rheumatoid arthritis, biochemical estimation, radiological examination.

ABSTRACT

Objective: To evaluate anti-arthritic potential of methanolic extract of rhizome of *Costus speciosus* Koen. **Methods:** The powdered drug was extracted with 80% methanol. The crude extract was subjected to phytochemical investigation and was evaluated for its anti-arthritic potential by freund's complete adjuvant induced arthritis model in adult wistar albino rats. Determination of different parameters like arthritic score, arthritic index, paw thickness, body weight, and pain, altered liver enzymes and biochemical estimation like, nitric oxide level and Plasma TNF-α level was done. Finally, radiological estimation and histopathology of tibio tarsal joints was performed. Statistical analysis was performed using one way ANOVA followed by Dunnet's test at different p-values. **Results:** Phytochemical study revealed the presence of flavonoids, phenolic compound, saponins and carbohydrates. For different parameter mentioned above, anti-arthritic activity shown by prophylactic high dose test extract (200 mg/kg) was as potent as standard drug Indomethacin (10 mg/kg). The effect of Prophylactic low dose extract (100 mg/kg) and therapeutic high dose extract (200 mg/kg) was less than that of Indomethacin (10 mg/kg). Furthermore, Therapeutic low dose extract (100 mg/kg) was not effective. Conclusions: The obtained results indicate that *Costus speciosus* rhizome extract possess significant anti-arthrtic potential.

INTRODUCTION

Currently, there is extensive utilization of higher plant as a major source of therapeutic agent throughout the world, as they contain many phytoconstituents of pharmacological benefits (Rani et al., 2012).Secondary plant metabolites previously with unknown pharmacological activities have been investigated as a source of medicinal agents (Momin et al., 2014). India has officially recognized list of 45,000 plant species among which list of 7500 plant species were suggested for their medicinal uses (Srivastava et al., 2011). The World Health Organization (WHO) estimates that 80% of the world population presently uses herbal medicine for some aspect of primary health care. Herbal medicine is a major component in all indigenous people's traditional medicine and a common element in Ayurvedic, homeopathic, naturopathic, traditional oriental. WHO notes that of 119 plant-derived pharmaceutical medicines, about 74% are used in modern medicine in ways that correlated directly with their traditional uses. Major pharmaceutical companies are conducting extensive research on plant materials gathered from the rain forests and other places for their potential medicinal value (Oristoki and Oguntibejw, 2010).

Rheumatoid arthritis (RA) is common auto-immune inflammatory arthritis, with prevalence of 0.5-1% occurring throughout the world and ethnic group with reduction in expected lifespan by 8-15 years (Boon, 2006, Srivastava et al., 2012). NSAIDS, DMARDS and combination of both are preferred medication for present scenario. But, adverse effect attributable to use of Disease modifying antirheumatic drugs (DMARDs) and Non-steroidal anti-inflammatory drugs (NSAIDS), particularly in aged patient, has dragged their attention for alternative medication (Bhangle and Acharya, 2014)

Costus speciosus is herbaceous plant belonging to family Costaceae (Chang et al., 2012). *Costus speciosus* has been scientifically possesses anti-inflammatory activity, anti-nociceptive activity, anti-diabetic activity, anti-oxidant activity, anti-microbial, normoglycemic, hypoglycaemic and oestrogenic activity.

*Corresponding Author
Email: salmanali.ali32@gmail.com

Traditionally, the rhizome extract of *Costus speciosus* is used in rheumatism and antiarthritic activity of aerial part of *Costus speciosus* is also reported. Anti-arthritic effect of rhizome of *Costus speciosus* are still to be explored. Therefore, the present research work has been undertaken to evaluate the anti-arthritic effect of rhizome extract of *Costus speciosus*.

MATERIALS AND METHODS

Collection and identification of plant material

The Semi-dried rhizome of *Costus speciosus* were identified, purchased and authenticated from VHCA herbals, Karnal, Haryana, India.

Extraction of rhizome of *Costus speciosus* rhizome

The dried powder of *Costus speciosus* koen rhizome was extracted using soxhlet apparatus with chloroform for 60 min to remove the wax, oil, chlorophyll, and non-polar phenolic compounds. Then, the residue was extracted with 80% methanol solution for 12 hrs at temperature 70 – 75 º C. The extract was concentrated under vacuum using rotary vacuum evaporator (Flash, Bucchi type), dried and weighed (Chang et al., 2012).

Experimental Animals

All the experiments were performed using male wistar albino rats weighing between 150-200 gm of either sex. Animals were procured from Central Animal House, MIET, Meerut. Animals were approved by Institutional Animal Ethic Committee (IAEC) of MIET, Meerut.

Approval number CPCSEA No. 711/02/a/CPCSEA was given for this work. Animals were housed in polypropylene cages. Standard conditions of temperature 25±2 °C and relative humidity 60-70% was maintained. The animals were fed with a standard diet and water *adlibitum*. Six animals were used in each group.

Drugs and Chemicals

Drugs

Indocap (Indomethacin) was purchased from local medical store of Meerut.

Chemicals

Freund's complete adjuvant was purchased from Sigma Aldrich. EDTA, was purchased from Rapid Diagnostic pvt. Ltd. Delhi, Sodium chloride, Zinc sulphate, barium chloride, Sulphuric acid, 5, 5-dithiobis-2-nitrobenzoic acid (DTNB), Acetyl thiocholine iodide, Trichloroacetic acid (TCA), Thiobarbituric acid (TBA), Sodium dihydrogen phosphate, disodium hydrogen phosphate, Petroleum ether, Ethanol, Chloroform, Methanol were purchased from Central Drug House Laboratory (CDH). Laboratory reagent grade chemicals were used in the study.

Phytochemical screening

Test for Flavanoids: Zinc hydrochloride test
Test for Saponins: Foam test
Test for Alkaloids: Dragendorff's Test
Test for Steroids and Sterols: Salkowski Test
Test for Amino Acids: Ninhydrin Test
Test for Carbohydrates: Molisch's Test (Treas and Evans, 2002)
Test for Tannins (Khandelwal, 2008): Extract + 5% ferric chloride solution
Test for phenolic compounds (Al-Mailiki, 2011): Ferric chloride test

Acute oral toxicity Study (LD_{50}):

Animals were fasted prior to test drug administration. Following the period of fasting animals was weighed and then the test substance administered in a single dose of 2000 mg/kg to animals by oral gavage. After the test drug administration, food was withheld for next 3-4 hours. Following administration, animals were closely observed for next 4 hours to see any clinical symptom, any change in behavior or mortality. After 6 hours of test administration the animals weighed again. A careful clinical examination was made once in each day for next 14 days (OECD 4/26, 2006)

Freund's complete adjuvant induced arthritis

Induction of arthritis

0.1ml of Freund's complete adjuvant (FCA) (composed of 1 mg/ml heat killed Mycobacterium tuberculosis, mineral oil and mannide monooleate) was injected in sub-plantar region of left hind paw of rats (Anderson, 2009).

Treatment Protocol

Rats were divided into seven groups. Group I served as control group and received 0.5% of CMC. Group II served as arthritic control and received 0.5% CMC and FCA. Group III served as standard group and received Indomethacin (10 mg/ml, p.o). Group IV and group V served as test group and received prophylactic low dose of test extract (100 mg/kg) and prophylactic high dose of test extract (200 mg/kg), respectively. Group VI and group VII served as test group and received therapeutic low dose of test extract (100 mg/kg) and therapeutic high dose of test extract (200 mg/kg). All the group except group I, received 0.1 ml of FCA along with treatments. All the treatments of group I and V were started at day 0 and continued till day 28. Treatments of group VI and VII were started at day 14 and continued till day 28.

Determination of antiarthritic potential

Arthritic Score

Animals were scored by observing their left hind paw from day 0 to day 28. Each animal was scored depending upon the severity of edema of paw. Score 0 indicated no arthritis. Score1 indicated redness or swelling of one toe/finger joint. Score 2 indicated redness and swelling of more than one toe/finger joint.

Score 3 indicated ankle and tarsal/metatarsal joint involvement. Score 4 indicated entire paw redness and swelling (Ramprasath, 2006).

Arthritic index

Arthritic index was calculated by sum of arthritic score (Vogel and Vogel, 2010).

Pain

Compression test

The digital vernier caliper measured the thickness of inflamed paw. The paw was compressed by rotating the screw of digital vernier caliper till pain was elicited as indicated by squeaking or leg withdrawal. The distance moved by the screw gauze was recorded (Sehgal
and Kumar, 2005).

Inflammation

Measurement of paw volume by plethysmometer

Paw volume was measured up to a fixed mark on the tibio tarsal joint at 0, 7, 14, 21 and 28 day using a manual plethysmometer. Edema volume was calculated at each time interval as the difference from paw volume at 0 day (Gaspar,2007).

Measurement of paw thickness

Paw thickness was measured by digital vernier caliper.

Body weight

Body weight was determined by digital weighing balance.

Determination of liver enzyme

Liver enzymes were determined by using commercial kit by autoanalyser.

Biochemical assay

Nitric Oxide estimation

About 1 ml of blood was drawn from the animals of each group by retro orbital puncture. The blood samples were centrifuged at 3000 rpm for about 10 min to collect the serum. Serum samples were taken in test tubes and treated with Griess reagent (1% sulphanilamide, 0.1% naphthyl-ethylenediamine dihydrochloride and 2.5% hydrochloric acid). The colorimetric reaction was allowed to proceed for 10 min at room temperature, and optical density was measured at 550 nm using a spectrophotometer. The concentrations of nitrite were calculated from a standard curve established with serial dilutions of sodium nitrite (Menaka et al., 2009.)

Plasma TNF-α estimation

As per the instruction of kit (Plasma TNF-α level, Sigma Aldrich, India).

Radiological estimation

Radiographic evaluation was performed by Kent diagnostic centre, Meerut.

Histopathological assessment

All the animals were sacrificed at the end of the study and tibio-tarsal joints of rats were removed and were sent to Deptt. of pathology, Swami Vivekanand Subharti University, Meerut for evaluation.

RESULTS

Phytochemical screening

Phytochemical test performed to determine the phytoconstituents revealed the presence of phenolic compound, flavonoids, steroidal compounds, saponins and carbohydrates.

Acute oral toxicity

No sign of mortality was found at the dose of 2000 mg/kg. Hence two doses were selected 100 mg/kg as lower dose and 200 mg/kg as higher dose to check the effectiveness of test drug.

Anti-arthritic activity

Arthritic score

The mean arthritic score in FCA induced arthritis and other treatment group was progressive till day 7. Treatment with Indomethacin (10 mg/kg, p.o) reduced the arthritic score significantly from Day 7 to Day 28. Similarly, in PHDE (200 mg/kg, p.o) there is significant reduction in arthritic score from Day 14 to day 28. In PLDE (100 mg/kg, p.o) reduction in mean score is less significant than Indomethacin and PHDE. In TLDE (100 mg/kg, p.o) and THDE (200 mg/kg, p.o) reduction in mean arthritic score is less significant as compared to all groups. The results are shown in Table 1.

Table 1: Effect of *Costus speciosus* Koen extract on arthritic score.

Treatment	Arthritic score				
	Day 0	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Control (0.5 % CMC)	0	0	0	0	0
Arthritic control	1.67 ±0.21	3.67 ± 0.21	3.67 ± 0.21	3.83 ± 1.66	3.50 ± 0.22
Indomethacin (10 mg/kg)	1.83 ± 0.17	2.16 ± 0.17***	1.67 ± 0.21***	1.34 ± 0.21***	0.83 ± 0.30***
PLDE (100 mg/kg)	1.85 ± 0.16	3.34 ± 0.33	2.67 ± 0.21	2.67 ± 0.21**	2.17 ± 0.30**
PHDE (200 mg/kg)	1.80 ± 0.16	2.84 ± 0.30	2.33 ± 0.21	1.83 ± 0.30***	1 ± 0.25***
TLDE (100 mg/kg)	1.84 ± 0.17	3.83 ± 0.16	3.67 ± 0.210	3 ± 0.25*	2.17 ± 0.17**
THDE (200 mg/kg)	2 ± 0	3.66 ±0.21	3.83 ± 0.166	2.67 ± 0.210**	1.67 ± 0.210***

Results are expressed as mean±sem, (n=6), analysed by one way ANOVA followed by Dunnet's test. * p<0.05, **p<0.01, ***p<0.001 when arthritic control compared with other treated groups.

Arthritic index

Arthritic index was found to be maximum in arthritic control group as compared to other treatment group. It was found to be 22 on day 7 and 21 on day 28 reflecting that there is no recovery from inflammation. Whereas in other treatment group arthritic index kept on decreasing weekly reflecting the best results in group receiving indomethacin (10 mg/kg, p.o) with arthritic index of 13 on day 7 and 5 on day 28 and in PHDE (200 mg/kg, p.o) arthritic index was found to be 17 on day 7 and decreased to 6 on day 28. The results revealed the protective effect of Indomethacin and PHDE (200 mg/kg). Reduction in arthritic index was also seen in PLDE (100 mg/kg) and THDE (200 mg/kg) but it was less significant.

Compression pain test

The effect of PHDE (200 mg/kg BW) was comparable to standard indomethacin, since, in both of these groups distance travelled (mm) by rotating screw gauze was maximum, among all other treatment groups.

In PHDE (200 mg/kg BW) group animals the pain threshold was significantly increased in comparison to animals in PLDE (100 mg/kg BW) group. In TLDE (100 mg/kg BW) and THDE (200 mg/kg BW) there were no relief from pain till second week but as soon as treatment was started pain threshold was increased but not as significant as of standard group. The results are shown in Table 2.

Inflammation

Measurement of paw volume by digital plethysmometer

Animals in treatment groups, Indomethacin (10 mg/kg BW) and PHDE (200 mg/kg BW) showed least volume of mercury displaced indicating highly significant reduction in paw edema when compared to arthritic control group. In therapeutic dose treatment groups, animals of TLDE (100 mg/kg BW) and THDE (200 mg/kg BW) volume of mercury displaced increased till two weeks. Since, the treatment started after two weeks of induction of arthritis. The results are shown in Table 3.

Percent inhibition of joint inflammation is maximum in group receiving Indomethacin (10 mg/kg BW) i.e. 55.63% followed by PHDE (200 mg/kg) with 51.1% of inhibition. Percent inhibition of PHDE (200 mg/kg) was followed by THDE (200 mg/kg) and PLDE (100 mg/kg) with 26.35 % and 21.24 % respectively. And, in TLDE (100 mg/kg) percent inhibition of inflammation was lowest 14.62%.

Average change in paw thickness (mm)

Paw thickness was maximum in animals of arthritic group indicating highest inflammation. Paw thickness of animals in standard group (Indomethacin 10 mg/kg BW) decreased significantly weekly showing highly significant anti-inflammatory effect. Animals in PHDE (200 mg/kg BW) group have comparable efficacy to standard, in reducing paw thickness. Treatment of animals with PLDE (100 mg/kg BW) also reduced paw thickness but less significantly. THDE (200 mg/kg BW) and TLDE (100 mg/kg BW) also reduced paw thickness but in later stage of the study, since, the treatment was started at 14 day after the induction of arthritis. The results are shown in table 4.

Body weight

Body weights of animals of standard group receiving Indomethacin (10 mg/kg BW) and PHDE (200 mg/kg BW) were increased significantly. In PLDE (100 mg/kg BW) increase in body weight was less significant. However, in TLDE (100 mg/kg BW) and THDE (200 mg/kg BW) there was increase in body weights as treatment started but it was not significant. Results are shown in table 5.

Table 2: Effect of *Costus speciosus* Koen extract on compression pain test.

Treatment	Compression pain test				
	Day 1	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Control (0.5 % CMC)	0.42 ± 0.013	0.42 ± 0.012	0.43 ± 0.01	0.43 ± 0.012	0.43 ± 0.01
Arthritic control	0.22 ± 0.013	0.20 ± 0.016	0.2 ± 0.014	0.13 ± 0.012	0.09 ± 0.006
Indomethacin (10 mg/kg)	0.20 ± 0.019	0.23 ± 0.015	0.28 ± 0.014**	0.34 ± 0.009***	0.41 ± 0.008***
PLDE (100 mg/kg)	0.22 ± 0.016	0.20 ± 0.018	0.22 ± 0.015	0.24 ± 0.014***	0.30 ± 0.007***
PHDE (200 mg/kg)	0.24 ± 0.019	0.28 ± 0.014*	0.31 ± 0.010 ***	0.35 ± 0.008***	0.39 ± 0.007***
TLDE (100 mg/kg)	0.23 ± 0.027	0.20 ± 0.024	0.168 ± 0.022	0.178 ± 0.019	0.218 ± 0.020***
THDE (200 mg/kg)	0.251 ± 0.029	0.215 ± 0.027	0.17 ± 0.024	0.185 ± 0.022	0.23 ± 0.026***

Results are expressed as mean±sem, (n=6), analysed by one way ANOVA followed by Dunnet's test. * P<0.05, **p<0.01, ***p<0.001 when arthritic control compared with other treated groups.

Table 3: Effect of *Costus speciosus* Koen extract on average change in paw volume in (ml).

Treatment	Average change in paw volume (ml)				
	Day 1	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Control (0.5 % CMC)	0.34± 0.01	0.42 ± 0.05	0.43 ± 0.05	0.43 ± 0.06	0.38 ± 0.09
Arthritic control	2.92 ± 0.17	3.29 ± 0.16	3.25 ± 0.07	3.15 ± 0.08	3.16 ± 0.10
Indomethacin (10 mg/kg)	2.93 ± 0.15	1.88 ± 0.10***	1.57 ± 0.09***	1.39 ± 0.05***	1.3 ± 0.04***
PLDE (100 mg/kg)	2.85 ± 0.09	2.8 ± 0.10**	2.78 ± 0.12**	2.63 ± 0.11**	2.23 ± 0.09**
PHDE (200 mg/kg)	2.68 ± 0.03	2.41 ± 0.06***	1.98 ± 0.04***	1.65 ± 0.03***	1.31 ± 0.03***
TLDE (100 mg/kg)	2.53 ± 0.10*	2.98 ± 0.04	3.00 ± 0.03	2.78 ± 0.03*	2.16 ± 0.02**
THDE (200 mg/kg)	2.58 ± 0.10**	2.93 ± 0.07*	2.86 ± 0.08**	2.48 ± 0.10**	1.9 ± 0.06***

Results are expressed as mean±sem, (n=6), analyzed by one way ANOVA followed by Dunnett's test. * P<0.05, **p<0.01, ***p<0.001 when arthritic control compared with other treated groups.

Table 4: Effect of *Costus speciosus* Koen extract on average change in paw thickness (mm).

Treatment	Average change in paw thickness (mm)				
	Day 1	Day 7	Day 14	Day 21	Day 28
Control (0.5 % CMC)	4.16±0.03	4.24 ± 0.05	4.24 ± 0.06	4.25 ± 0.05	4.21 ± 0.05
Arthritic control	7.73 ± 0.25	8.06 ± 0.12	8.08 ± 0.03	8.02 ± 0.04	7.96 ± 0.09
Indomethacin (10 mg/kg)	7.79 ± 0.20	5.93 ± 0.16***	5.7 ± 0.15***	5.44 ± 0.14***	5.38 ± 0.09***
PLDE (100 mg/kg)	7.67 ± 0.15	7.61 ± 0.14*	7.38 ± 0.26**	7.22 ± 0.20**	6.67 ± 0.23***
PHDE (200 mg/kg)	7.98 ± 0.08	7.27 ± 0.09***	6.23 ± 0.05***	5.98 ± 0.02 ***	5.49 ± 0.09***
TLDE (100 mg/kg)	7.39 ± 0.22	7.87 ± 0.12 ^{ns}	7.97 ± 0.03 ^{ns}	7.49 ± 0.12*	6.52 ± 0.02***
THDE (200 mg/kg)	7.22 ± 0.22	7.9 ± 0.07^{ns}	7.76 ± 0.11 ^{ns}	7.1 ± 0.21***	6.08 ± 0.06***

Results are expressed as mean±sem, (n=6), analysed by one way ANOVA followed by Dunnet's test. * P<0.05, **p<0.01, ***p<0.001 when Arthritic control group compared with other treated groups.

Table 5: Effect of *Costus speciosus* Koen extract on body weight of animals.

Treatment and dose	Body weights					% change in body weight
	Day 0	Day 7	Day 14	Day 21	Day 28	
Control (0.5 % CMC)	211±15.85	211.33±16.59	212.16±14.56	213.33±14.14	214±13.95	**1.92±1.39** ↑
Arthritic control	191.16±6.52	174.5±7.23	170.33±5.74	162.5±5.16	154.33±4.65	**19.14±1.31** ↓
Indomethacin (0.3 mg/kg)	220.5±3.20	209.16±3.38	219.83±3.32	233.66±3.34	238.83±3.43	**8.29±0.39** ↑***
Prophylactic low dose (100 mg/kg)	137.5±2.813	127.5±2.90	133.83±2.89	137.83±3.38	139.66±3.26	**1.53±0.53** ↑***
Prophylactic high dose (200 mg/kg)	153.5± 2.55	147.33± 2.56	151.5± 2.61	155.66±2.96	160±2.75	**4.23±0.55** ↑***
Therapeutic low dose (100 mg/kg)	121.16±5.52	104.66±5.15	90.33±5.19	96.33±5.70	105.5±6.46	**11.26± 3.10** ↓
Therapeutic high dose (200 mg /kg)	129.83±1.57	115.66±2.15	105.66±2.85	117.5±3.34	125.66±2.72	**3.25±1.07** ↓

Results are expressed as mean±sem, (n=6), analysed by one way ANOVA followed by Dunnet's test. * P<0.05, **p<0.01, ***p<0.001 when Arthritic control group compared with other treated groups.

Table 6: Effects of *Costus speciosus* Koen extract Liver enzymes.

Treatment	Liver enzymes		
	SGPT (U/L)	SGOT (U/L)	ALP (U/L)
Control (0.5 % CMC)	49.09 ± 0.76	56.56 ± 2.00	131.70 ± 1.20
Arthritic control	138.85 ± 3.36	143.16 ± 2.35	256.32 ± 4.60
Indomethacin (10mg/kg)	74.97 ±0.98***	77.63 ± 0.80***	159.66 ± 1.84***
PLDE (100 mg/kg)	130.45 ± 1.14**	131.32 ± 1.88**	239.00 ± 1.30***
PHDE (200 mg/kg)	91.66 ± 1.3***	93.63 ± 3.75***	186.59 ± 1.24***
TLDE (100 mg/kg)	134.57 ± 1.4	139.66 ± 0.91	249.166 ± 2.55
THDE (200 mg/kg)	112.94 ± 1.10***	119.53 ± 3.09***	216.93 ± 1.71***

Results are expressed as mean±sem, (n=6), analysed by one way ANOVA followed by Dunnet's test. * P<0.05, **p<0.01, ***p<0.001 when Arthritic control group compared with other treated groups.

Determination of liver enzyme

Elevation of liver enzymes ALP, SGOT, SGPT generally occurs in arthritis. There were marked elevations of liver enzymes revealing the progression of disease in animals of arthritic group. In standard group (Indomethacin 10 mg/kg BW) and PHDE (200 mg/kg BW) group there was significant decrease in level of these enzymes. In PLDE (100 mg/kg BW) and THDE (200 mg/kg) the decrease is less significant when compared to arthritic control. However, in TLDE (100 mg/kg BW) there was no alteration in level of liver enzymes. Results are shown in table 6.

Estimation of nitric oxide

The changes in nitric oxide concentration assayed in serum of rats at 28th day are presented in figure 1. The result has shown that there is significant increase in level of nitric oxide in arthritic control group. It can easily be found out with help of figure that there is significant reduction in level of nitric oxide in standard indomethacin group, PHDE (200 mg/kg), PLDE (100 mg/kg), and THDE (200 mg/kg). However, the reduction of nitric oxide level in TLDE (100 mg/kg) is not significant.

Estimation of plasma TNF-α

Figure 2 clearly indicate that high level of TNF-α were observed in arthritic control group rats. Indomethacin, PHDE (200 mg/kg), PLDE (100 mg/kg) and THDE (200 mg/kg) reduced the level of plasma TNF-α significantly. However, the effect of TLDE (100 mg/kg) is not significant.

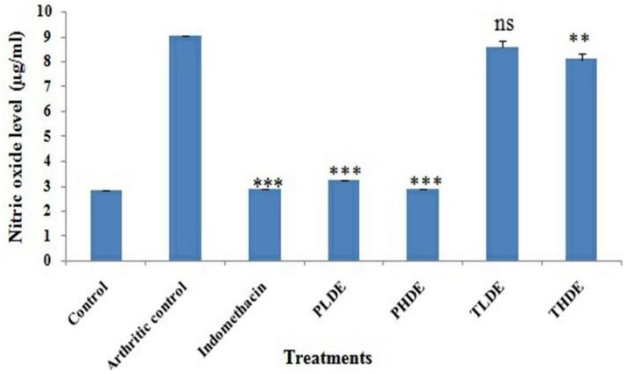

Fig. 1: Effect of *Costus speciosus* on Nitric oxide level (µg/ml) in treatment groups. Results are expressed as mean±SEM, (n=6), analyzed by one way ANOVA followed by Dunnet's test. * p<0.05, **p<0.01, ***p<0.001 when arthritic control compared with other treated.

Radiological evaluation

Radiological examination of tibio-tarsal joint was done. It was seen that normal control group animals showed normal picture of joint. Animal in arthritic control group showed diffused joint and narrowing of the joint space. Animals in standard group

(Indomethacin (10 mg/kg)) and other treatment group like PLDE (100 mg/kg), PHDE (200 mg/kg), and THDE (200 mg/kg) shows significant reduction in narrowing of joint space whereas, in TLDE (100 mg/kg) the effect was less significant in reduction in narrowing of joint space.

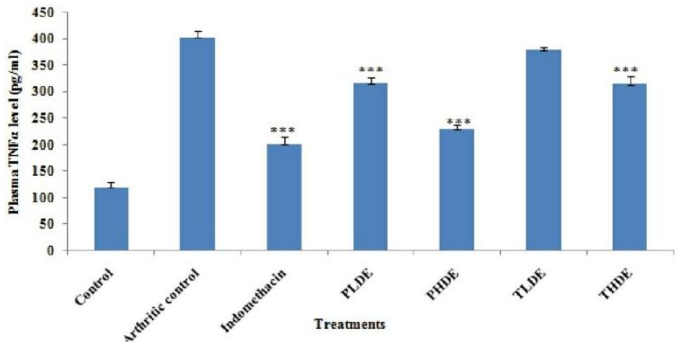

Fig 2: Effect of *Costus speciosus* on plasma TNF-α level (pg/ml) in treatment groups. Results are expressed as mean±SEM, (n=6), analyzed by one way ANOVA followed by Dunnet's test. * p<0.05, **p<0.01, ***p<0.001 when arthritic control compared with other tre

Histopathological assessment

Histopathology study of tibio-tarsal joint was also done. In Normal control group, joint of animal showed normal cross section of joint. In Arthritic control group, joint of animal showed marked synoviocytes proliferation and inflammation with eosinophils and plasma cell. Joint of animal in PLDE (100 mg/kg) shows acute and chronic inflammation of joint space. Marked proliferation of synoviocytes. PHDE (200 mg/kg) joint showed normal minimal inflammation and reduced inflammatory mediator and less synoviocytes proliferation. In TLDE (100 mg/kg) crossection of complete joint shows marked synovial proliferation and sparse to moderate mixed amount of inflammatory infiltrate. In THDE (200 mg/kg) showed mild synovial proliferation. Inflammation is mostly chronic.

DISCUSSION

A small portion of methanolic extract was used for phytochemical evaluation which confirmed the presence of Flavonoids, phenolic compounds, steroidal compound, saponin and carbohydrates. It was found out in previous research that extracts of *Costus speciosus* in different polar solvents were rich in polyphenols(Nehete et al., 2010). Polyphenols reduces the chronic inflammation or its downstream consequences. They could reduce various pro-inflammatory substance productions through anti-oxidant effect (Ghiringhelli et al., 2012).The pro-inflammatory cytokines/chemokines such as TNFα, IL-1β, IL-6, IL-8 are also reduced in many cell types. Reactive oxygen species are known plays an important role in development and maintenance of rheumatoid arthritis in human and animal models. Synoviocytes and chondrocytes produce nitric oxide which is responsible in production free radical giving rise to highly toxic radical peroxynitrite. The study of experimental animals has demonstrated increased activity of iNOS. Inhibition of NF-κB prevents reactive oxygen species by inhibition iNOS. Polyphenols inhibits NO release by suppressing NOS activity. Varieties of flavonoids suppress NO production and inhibition of NOS transcription, which occur by binding of NF-κB to promoter of iNOS thereby in activating them (Santangelo et al., 2007, Cheeke et al., 2006). The presence of flavon-3-ols has been confirmed scientifically in *Costus speciosus*. Three of flavonoids such as quercitin and rutin and quercitrin were known to possess very high anti-oxidant activity (Chang et al., 2012). It has also been reported that two of the constituent of *Costus speciosus*, Costunolide and eremanthin could significantly reduced TBARS level and increase GSH level (Elisa et al., 2010). This anti-oxidant activity could also be the possible mechanism of anti-arthritic effect of *Costus speciosus*. It was reported in different research that rhizome of *Costus speciosus* possess anti-inflammatory, anti-oxidant, antipyretic, activities. So, the observed anti-arthritic activity of *Costus speciosus* is may be due to its anti-oxidant potential via different mechanisms. Based on these observations we reached to a conclusion that methanolic extract of *Costus speciosus* was found to be rich in flavonoid, phenolic compounds, carbohydrates and steroidal compounds. The presence of these compounds undertake role in antiarthritic activity of rhizome of *Costus speciosus*. The antiarthritic activity was demonstrated by measurement of arthritic score, arthritic index, paw thickness, inflammation, and pain. Measurement of liver enzymes ALP, SGOT, SGPT was also done because elevation of liver enzymes generally occurs in arthritis. There were marked reductions of liver enzymes indifferent treatment groups. Anti-arthritic effect attributable to anti-oxidant and anti-inflammatory property was confirmed by marked reduction in nitric oxide level, plasma TNF-α level, Rheumatoid factor level and C-reactive protein level. Finally, radiological estimation and Histopathological estimation of tibio tarsal joint revealed the anti-arthritic potential of methanolic extract of rhizome of *Costus speciosus*. Finally it can be concluded that, additional works on identification and isolation of active constituents in the extracts may be explored to determine the exact mechanism of the antiarthritic activity.

CONCLUSION

In conclusion, the extract is effective in reducing ill-effects of RA both prophylactically and therapeutically. But, therapeutic low dose extract (TLDE 100 mg/kg) does not show significant effect. While, PHDE (200 mg/kg), PLDE (100 mg/kg) and THDE (200 mg/kg) showed significant reduction in arthritic score, arthritic index, pain, edema and inflammation. They also, normalized the body weight of arthritic animal and reduced liver enzymes. From biochemical estimation like nitric oxide level and TNF-α level it was clear that these prophylactic and therapeutic doses are capable of reducing inflammatory mediators. Finally radiological and histopathological analysis supported the anti-arthritic profile of *Costus speciosus* rhizome extract. Among all these treatment doses, PHDE (200 mg/kg) afforded maximum protection against FCA induced arthritis.

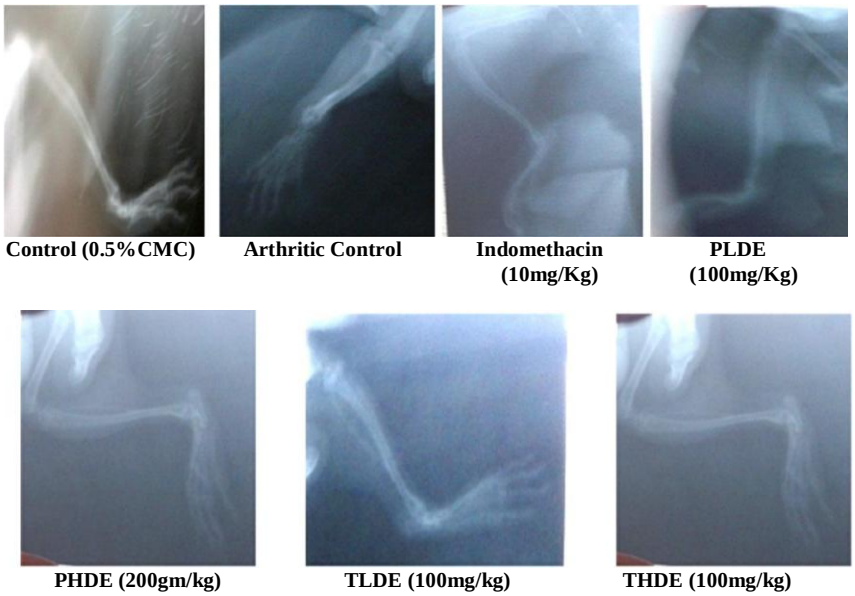

Fig. 3: Radiological examination of Tibio-tarsal joints of animals in different treatment groups.

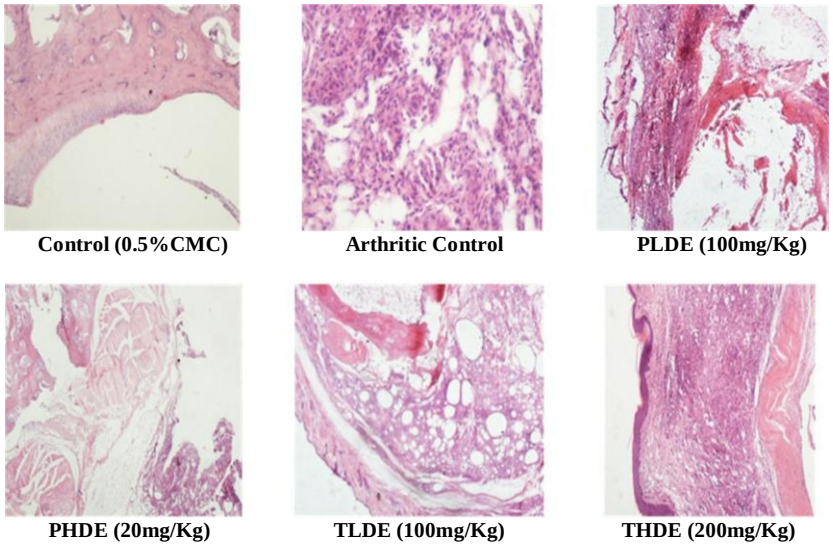

Fig. 4: Histopathological study of tibio-tarsal joint of animals in different treatment groups.

REFERENCES

Al-Mailiki ADM. Isolation and identification of phenolic compounds from *Elettaria cardamomum* seeds and study of their medicinal activity against Pathogenic Bacteria of Prostate Gland. Journal of Misan Researches, 2011; 8(15): 13-35.

Anderson ML. Evaluation of acute and chronic treatments with *Harpagophytum procumbens* on Freund's adjuvant-induced arthritis in rats. J Ethnopharmacol, 2004; 91: 325–330.

Bhangle J, Acharya S. Antiarthritic activity of *Cynodon dactylon* (L.) Pers. Indian J Exp Biol, 2014; 52:215-222.

Boon NA, Colledge NR. 2006. Walker Brian. Davidson's principle and practice of medicine. 20th ed. Churchill livingtone: Elsevier.

Chang YQ, Tan SN, Yong JWH, Ge L. Determination of flavonoids in *Costus speciosus* and etlingera elatior by liquid chromatography-mass spectrometry. Analyt Lett, 2012; 45: 345–355.

Cheeke PR, Piacente S, Oleszek W. Anti-inflammatory and anti-arthritic effects of yucca schidigera: A Review. J Inflamm, 2006; 3(6):1-7

Elisa J, Daisy P, Ignacimuthu S. Antioxidant activity of costunolide and eremanthin isolated from *Costus speciosus* (Koen ex. Retz) Sm. Chem Biol Interact, 2010; 182(1):67-72

Gaspar MM. Enzymosomes with surface-exposed superoxide dismutase: In vivo behaviour and therapeutic activity in a model of adjuvant arthritis. J Control Release, 2007; 117:186–195.

Ghiringhelli F, Rebe C, Hichami A, Delmas D. Immunomodulation and anti-inflammatory roles of polyphenols as anticancer agents. Anticancer Agents Med Chem, 2012; 9(3): 852-73

Khandelwal KR. 2008. Practical Pharmacognosy. New Delhi: Pragati Books Pvt. Ltd

Menaka KB, Ramesh A, Thomas B, Kumari N.S. Estimation of nitric oxide as aninflammatory marker in periodontitis. JISP, 2009; 13(2).

Momin MAA, Bellah SF, Rahman SMR, Rahman AA, Murshid GMM, Emran TB. Phytopharmacological evaluation of ethanol extract of *Sida cordifolia* L. roots. Asian Pac J Trop Biomed, 2014; 4(1): 18-24.

Nehete J, Bhatia M, Narkhede M. In-vitro estimation of antioxidant activity and phenolic content of *Costus speciosus* (Koen) J.E. Sm. Iran J Pharm Res, 2010;9(3): 271-277.

OECD guideline for the testing of chemicals. Acute oral toxicity-Up- and- down procedure (UDP). 2006; 4/26.

Orisatoki RO, Oguntibeju OO. The role of herbal medicine use in HIV/AIDS treatment. ACMicrob, 2010; 1(3.3).

Ramprasath VR. Curative effect of *Semecarpus anacardium* Linn. nut milk extract against adjuvant arthritis—with special reference to bone metabolism. Chem-Biol Interact, 2006; 160:183– 192.

Rani SA, Sulakshana G, Patnaik S. *Costus speciosus*, an antidiabetic plant – review. FS J Pharm Res, 2012; 1(3): 52-53.

Santangelo C, Vari R, Scazzocchio B, Benedetto RD, Filesi C, Masella R. Polyphenols, intracellular signaling and inflammation. Ann Ist Super Sanita, 2007; 43(4): 394-405.

Sehgal R, Kumar VL. *Calotropis procera* Latex-Induced Inflammatory Hyperalgesia—Effect of Anti-inflammatory Drugs. Mediators Inflamm, 2005; 4:216–220.

Srivastava S, Singh P, Mishra G, Jha KK, Khosa RL. *Costus speciosus* (Keukand): A review. Der Pharmacia Sinica, 2011;2(1):118-128

Srivastava S, Singh P, Mishra G, Jha KK, Srivastava S, Khosa RL. Evaluation of antiarthritic potential of methanolic extract aerial parts of *Costus speciosus*. J Ayurveda Integr Med, 2012; 3(4): 204-208.

Trease GE, Evans WC. 2002. A Text book of Pharmacognosy. 15th ed. Harcourt brace & Co. Asia, Pvt. Ltd: W.B. Saunders Company Ltd.

Vogel HG, Vogel, WH. 2010. Drug Discovery and Evaluation. Pharmacological Assay. Mass J. Springer.

How to cite this article:

Chandra Kala, Syed Salman Ali, Mohd Abid, Uma Shanker Sharma, Najam Ali Khan. Evaluation of in-vivo antiarthritic potential of methanolic extract of *Costus speciosus* rhizome. J App Pharm Sci, 2015; 5 (08): 046-053.