Proceedings S.Z.M.C. Vol: 36(1): pp. 26-30, 2022. PSZMC-829-36-1-2022

Colebrookea Oppositifolia Anti Arthritic Potential Vs Methotrexate in Pristane Induced Rat Arthritis

[1]Maheen Fatima, [2]Moneeb Ashraf, [3]Urooj Fatima, [4]Muhammad Imran, [1]Amer Hassan Siddiqui, [5]Tafzeel Fatima

[1]Department of Pharmacology, Post Graduate Medical Institute, Lahore
[2]Department of Pharmacology, King Edward Medical University, Lahore
[3]Department of Pharmacology, Fatima Memorial Hospital, Lahore
[4]Department of Histopathology, Jinnah Hospital, Lahore
[5]Department of Medicine, Sir Ganga Ram Hospital, Lahore

ABSTRACT

Introduction: Rheumatoid arthritis (RA) is a chronic inflammatory disease of joints with 0.24% global prevalence. Numerous pharmacological agents are available for the management of RA, but they are associated with many adverse effects. Alternative therapies for disease management are urgently needed. *Colebrookea oppositifolia (CO)* is an important herb with various traditional uses and pharmacological actions. In the present study, anti-arthritic effect of ethanolic extract of *Colebrookea oppositifolia* (EECO) leaves was evaluated in pristane induced arthritic rats (PIA) and compared to methotrexate by assessing body weight, clinical score of inflammation and histopathology.

Aims & Objectives: To study anti-arthritic effect of *C. oppositifolia* on a rat model of pristane induced arthritis.

Place and duration of study: This experimental study was conducted in Animal House of Post Graduate Medical Institute, Lahore from March to September 2019.

Material & Methods: Total forty female Sprague Dawley rats were categorized into five equal groups (n=8). Group A (normal control), group B (disease control). Group C and D belonged to low dose (250mg/kg) and high dose (500mg/kg) EECO treated groups respectively, while group E was methotrexate treated group. Arthritis was induced within two weeks by single intradermal injection of pristane on day 0 in groups B, C, D and E. At Day 15, treatment was initiated and at day 28 paw joint sections were taken for histopathology. Data input and analysis was done by using IBM SPSS version 24. p value ≤0.05 was considered significant.

Results: At week 4, significant increase (approximately 16%) in body weight was observed in all treated groups as compared to disease control. A significant reduction (more than 50%) in clinical score of arthritis was observed in all treated groups compared to diseased control group in which clinical score was 14.50±0.2. All extract and MTX treated groups showed significant improvement ($p<0.001$) in total histological score of arthritis (no rat was having severe disease) as compared to disease control group (75% of the rats were having severe disease).

Conclusion: This study supported anti-arthritic effect of EECO as illustrated by reduction in inflammatory and histopathological score.

Key words: Ethanolic extract of *Colebrookea oppositifolia* (EECO), *Colebrookea oppositifolia* (*C0*), anti-arthritic activity, pristane induced arthritic rats (PIA), histopathology.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic disorder predominantly affecting synovial joints, with global prevalence 0.24%.[1] RA being an autoimmune disease is associated with various immune cells (T cells, macrophages, fibroblasts and B cells) contributing to joint damage. The main histopathological changes in RA include abnormal proliferation of fibroblasts and synovial cells, resulting in thickening of synovial membrane, which progressively results in erosion of underlying structure of joint.[2] RA cannot be cured completely, however, recommended treatment approach is 'Treat to target approach', the objective of which is to achieve either remission or to lower disease activity.[3]The medical management of RA includes non-steroidal anti-inflammatory drugs (NSAIDS), steroids, disease modifying anti rheumatic drugs (DMARDs) and biological agents, depending on duration as well as severity of the disease process.[1] Among the DMARDs, methotrexate (MTX) acts as an anchor drug, as it is used alone or can be combined with other drugs.[4] However, its extensive use is associated with various detrimental side effects. Apart from the adverse effects, patient's expenditure on the management of the complications associated with treatment is also an issue of concern.[5]

Colebrookea oppositifolia (*C0*) is an important herb with various traditional uses and pharmacological actions.*C0* possess anti-inflammatory,[6] anti-oxidant,[7] cardio-protective,[8] anti-microbial,[9] and anti-ulcer[10] activities because of its rich flavonoids contents. The current study was conducted to evaluate the anti-arthritic activity of *C0* in pristane induced arthritic (PIA) rats.

MATERIAL AND METHODS

This experimental study was conducted in Animal House of Post Graduate Medical Institute, Lahore from March to September 2019, after approval by ethical committee of PGMI vide letter number 1137/EC/PGMI/2019.

Drugs and reagents: Pristane (Sigma), Ethanol (Merk) and Methotrexate (Howards) were purchased from local market.

Collection and Extraction of *C0*: Collection was done from Haripur, Hazara located in Khyber Pakhtunkhwa, province of Pakistan and authentication was done by Botany Department of GCU, Lahore vide voucher number Herb.Bot.3636. Shade dried 100g coarsely powdered leaves were dipped in 80% ethanol at 1:10 ratio for 72 hours, followed by filtration and evaporation via a rotary evaporator, yielding 20g of concentrated greenish brown, sticky, semisolid extract, which was freeze dried afterwards.[11]percentage yield of extract was 20%. EECO was readily soluble in distilled water forming homogenous solution.

Animals: Total 40 female Sprague Dawley rats at 6-8 weeks of age (120-150g) were taken and acclimatized according to the standard laboratory conditions for 7 days with free access to water and regular rat diet.

Grouping Experimental animals were randomly divided into five equal groups (A-E); with eight animals per group.

Establishment of pristane induced arthritis: Arthritis was induced within 2 weeks in all animals of group B, C, D and E by single intradermal injection of 0.5ml pristane at the tail base on day 0.[12]

Group	Intradermal injection at day 0	Oral treatment from 2-4 weeks (14-28 days)
A	0.5ml normal saline	Distilled Water 1ml
B	0.5ml pristane	Distilled Water 1ml
C	0.5ml pristane	250 mg/kg EECO
D	0.5ml pristane	500 mg/kg EECO
E	0.5ml pristane	1 mg/kg MTX

Table-1: Experimental groups for induction and treatment of rats.

Evaluation of body weight and clinical score of inflammation:

Each animal was weighed on day 0, followed by weekly measurement for next 4 weeks.

Clinical scoring of joint inflammation was performed on alternate days on all four limbs.

Arthritic score ranged from 0 to 4. Absence of swelling or redness =0, Swelling and/or redness in single joint (digit or paw) =1, in two joints=2, in more than two joints=3 and 4 =whole paw and digits involved.[13]

Histopathological examination of ankle joints:

At the end of study, on 4th week all animals were euthanized. Tissue specimen was examined under microscope after hematoxylin-eosin staining.

Histopathological scoring[14] was done by the following criteria:

Scoring for infiltration by mononuclear cells: 0=absent, 1= mild, 2= moderate, 3= severe.

Scoring for hyperplastic synovial cell infiltration: 0=absent, 1=mild (involvement of 1-3 layers), 2=moderate (involvement of 4-6 layers), 3=severe (involvement of 7 or more layers).

Scoring for villous hyperplasia: 0=absent, 1=few, short and scattered hyperplastic villi, 2= marked finger liked villi, 3=marked and diffused hyperplastic villi.

Scoring for pannus formation: 1=absent 2=mild pannus formation, 3=moderate synoviocytes proliferation and cartilage or bone invasion, 4=severe synoviocytes and inflammatory cell invasion into the cartilage or bone.

Total scoring for arthritis: Total scoring for arthritis was done by sum of above mentioned four histopathological parameters. 0=absent, 1-3=mild, 4-6 =moderate, more than 6= severe arthritis.

Statistical analysis:

Data input and analysis was done by using IBM SPSS version 24. As the data was normally distributed (tested by Shapiro Wilk test) ANOVA and post hoc Tukey's tests were used to test significance among all groups. Statistically significance was considered when p value was ≤ 0.05.

RESULTS

Body Weight

Comparison among groups at week 2, have shown significant lowering ($p \leq 0.001$) in mean weight in all disease induced groups in comparison to group A. Non-significant difference existed amongst all other groups at the end of week 2.

At week 4, mean body weight of groups A, C, D and E was significantly high ($p \leq 0.001$) in comparison to group B.

Week/ Group	week 0	week 2	week 4
A	122.2±0.6	140.0±1.5	153.2±1.8
B	123.5±0.	114.8±1.0	109.6±1.0
C	122.3±1.0	115.1±1.0	131.5±0.9
D	123.8±1.3	115.3±1.0	120.7±2.1
E	124.3±1.5	115.3±1.5	130.2±1.2

Table-2: Effect on body weight of rats (g) by *C0* and methotrexate in PIA (n=8).

Clinical Score

At week 2, significant difference ($p \leq 0.001$) was observed in mean clinical scoring of disease induced groups in comparison to group A and there was non-significant difference among all other groups. While, at week 4, significant difference ($p \leq 0.001$) existed in groups A, C, D, and E in comparison to group B. Similarly, significant difference also existed in groups A, B, C and D as compared to group E.

Week/Group	week 2	week 4
A	0.00	0.00
B	13.75±0.4	14.50±0.2
C	13.87±0.3	8.87±0.2
D	13.87±0.3	7.37±0.3
E	13.75±0.3	3.87±0.2

Table-3: Effect on clinical scoring of rats by *CO* and methotrexate in PIA (n=8).

Histopathological Scoring

Pair wise comparison for total arthritic score indicated that significant difference($p<0.01$) was present in total scoring of groups A, C, D &E in comparison to group B.

Total Histopathological Scoring	A	B	C	D	E
	%	%	%	%	%
Normal	100	0	0	0	0
Mild	0	12.5	50	100	87.5
Moderate	0	12.5	50	0	12.5
Severe	0	75	0	0	0

Table-4: Effect on histopathological scoring of rats by *CO* and methotrexate in PIA (n=8).

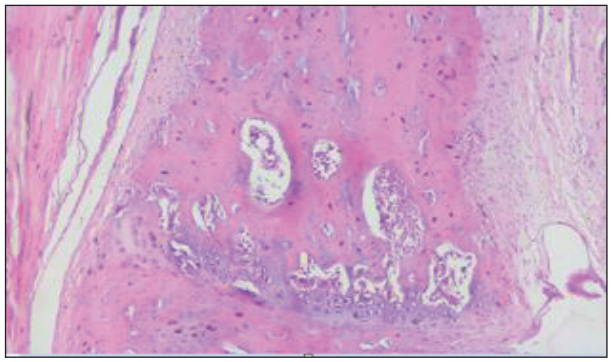

Fig-1: Group-A (Normal Control). Joint of normal healthy rats.

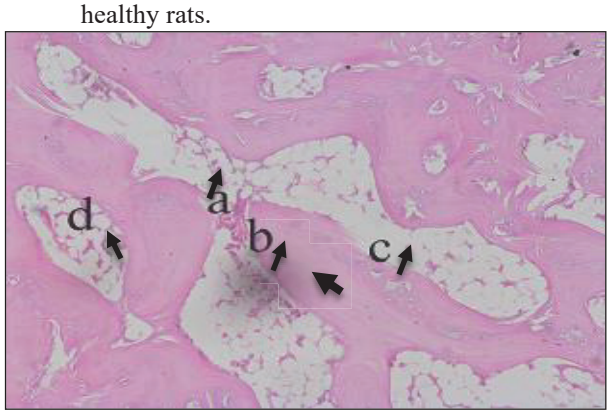

Fig-2: Group B (Disease Control), **a** & **c** shows bone erosion and pannus formation, **b** & **d** shows inflammatory cell infiltration.

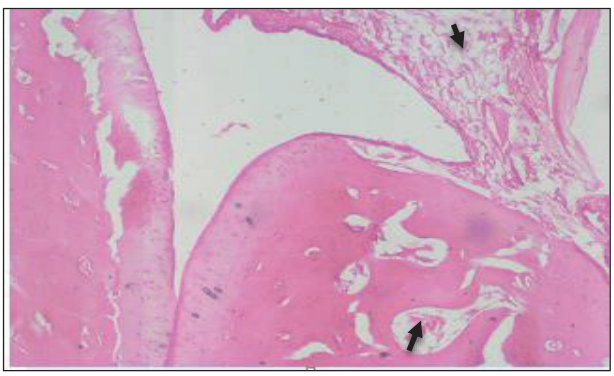

Fig-3: Group C (Low dose EECO). Treatment with low dose extract shows less infiltration by inflammatory cells.

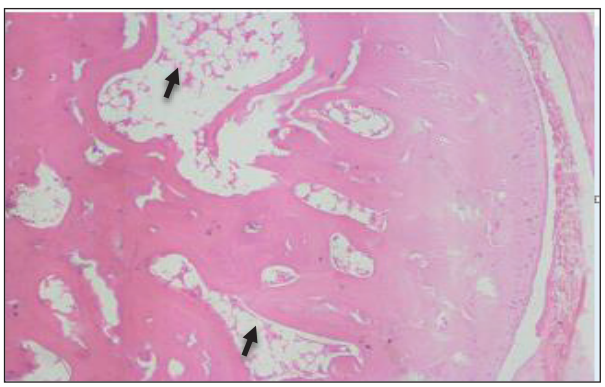

Fig-4: Group D (High dose EECO). High dose treatment of extract recovery of joints.

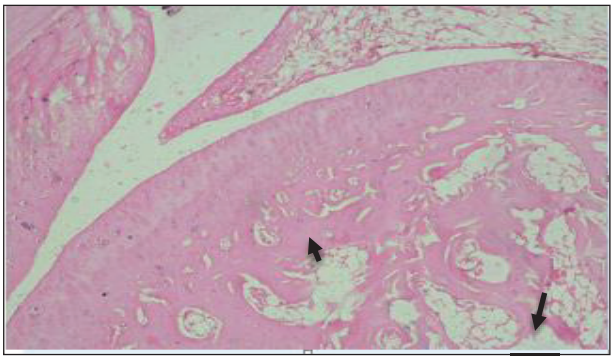

Fig-5: Group E (MTX treated). Reduced inflammatory cells infiltration and decreased synovial and villous hyperplasia.

Recovery of joint with reduced inflammatory cells infiltration and decreased synovial and villous hyperplasia in groups C, D and E.

DISCUSSION

Arthritis was induced by single pristane injection and its confirmation was done by observing signs of inflammation (swelling or redness) in all four limbs and by histopathological scoring.

Body weight declined significantly ($p<0.001$) in all disease induced groups till week 2 with almost 6% decline in body weight in contrast to normal control. While, at week 4 it significantly increased ($p<0.001$) in all extract and methotrexate treated groups approximately 16% in comparison to disease control group. Comparison of increase in body weight of rats between MTX treated group, low dose EECO extract treated group and high dose EECO extract treated group has shown a similar gain in body weight of rats. Similar pattern of restoration of body weight in arthritic animals has also been observed in previously conducted studies.[14,15]

At week 2, the clinical score for arthritis increased significantly ($p<0.001$) in all disease induced groups (group B, C, D and E) compared to group A, and at week 4, it significantly decreased in all extract and methotrexate treated groups in comparison to disease control group showing anti-arthritic activity of MTX and *C. oppositifolia*. These results are similar to previous studies, where significant reduction in clinical scoring of arthritic rats was observed by the use of medicinal plants having phytochemicals (aglyconic and glycosylated flavonoids, phenolic compounds and glycosides like acteoside) similar to that of *C. oppositifolia*.[16,17,18]

Significant reduction ($p<0.01$) in total histopathological scoring was noted in all treated groups in comparison to disease control group where decreased cell infiltration and reduced synovial hyperplasia was observed owing to anti-arthritic activity of MTX and *C. oppositifolia*. These results of histopathology are similar to previously conducted studies of plants having a glycolic and glycosylated flavonoids where reduction in cell infiltration and synovial hyperplasia has led to their anti-arthritic activity.[19,20]

Leaves of *CO* are locally used as decoction in northern areas of Pakistan for the treatment of rheumatism.[21] But no scientific data was available regarding the effectiveness of *CO* in the treatment of RA. This research was conducted for evaluating the effect of *CO* in arthritic rats. Recently performed study has shown that EECO has anti-arthritic potential owning to its rich flavonoids contents. This anti-arthritic action is due to its cytotoxic activity and inhibition of pro-inflammatory transcription factors NF-KB (nuclear factor kappa-light-chain-enhancer of activated B cells) and AP-1 (Activator protein 1).[6]

CONCLUSION

Results of the conducted study have shown that *CO* has anti-arthritic effects in PIA in rats, which is comparable to that of methotrexate. Further studies are required in order to identify various active principles of *CO* that have potential anti-arthritic potential.

REFERENCES

1. Littlejohn EA, Monrad SU. Early diagnosis and treatment of rheumatoid arthritis. Primary Care: Clinics in Office Practice. 2018; 45(2):237-55.
2. Mateen S, Zafar A, Moin S, Khan AQ, Zubair S. Understanding the role of cytokines in the pathogenesis of rheumatoid arthritis. Clinica Chimica Acta. 2016; 455:161-71.
3. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham III CO, et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis & rheumatism. 2010; 62(9):2569-81.
4. Arora S, Rafiq A, Jolly M. Management of rheumatoid arthritis: Review of current guidelines. Journal of Arthroscopy and Joint Surgery. 2016; 3(2):45-50.
5. Capron J, De Leonardis F, Fakhouri W, Burke T, Rose A, Jacob I. The impact of disease duration and disease activity on the cost of rheumatoid arthritis: results from Burden of Rheumatoid Arthritis Across Europe a Socioeconomic Survey (BRASS). Value in Health. 2017; 20(9):A532.
6. Peron G, Hošek J, Prasad Phuyal G, Raj Kandel D, Adhikari R, Dall'Acqua S. Comprehensive Characterization of Secondary Metabolites from Colebrookea oppositifolia (Smith) Leaves from Nepal and Assessment of Cytotoxic Effect and Anti-Nf-κB and AP-1 Activities In Vitro. International Journal of Molecular Sciences. 2020; 21(14):4897.

7. Subba B, Basnet P. Antimicrobial and antioxidant activity of some indigenous plants of Nepal. Journal of Pharmacognosy and Phytochemistry.2014; 3(1):62-67
8. Pallab K, Kush B, Kumar P, Girraj T, Kishor T, Singh N, et al. In vitro–in vivo evaluation of cardioprotective effect of the leaf extract of Colebrookea oppositifolia Sm. J Global Trend Pharm Sci. 2011; 2:310-24.
9. Mahapatra SK, Mookerjee M, Roy DS, Karak P, Das S, Dastidar SG. Evaluation of antimicrobial potentiality of a flavonoid, isolated from the leaf of the plant Colebrookea Oppositifolia. Int J Biol Pharm Res. 2013; 4:225-30.
10. Ghaisas M, Sharma S, Ganu G, Limaye R. Antiulcer activity of *Colebrookea oppositifolia Sm.* Research Journal of Pharmacology and Pharmacodynamics. 2010; 2(1):66-70.
11. Azwanida N. A review on the extraction methods use in medicinal plants, principle, strength and limitation. Med Aromat Plants. 2015;4(196):2167-0412
12. Tuncel J, Haag S, Hoffmann MH, Yau AC, Hultqvist M, Olofsson P, et al. Animal models of rheumatoid arthritis (I): pristane-induced arthritis in the rat. PLoS One. 2016; 11(5):e0155936.
13. Gul A, Kunwar B, Mazhar M, Faizi S, Ahmed D, Shah MR, et al. Rutin and rutin-conjugated gold nanoparticles ameliorate collagen-induced arthritis in rats through inhibition of NF-κB and iNOS activation. International Immunopharmacology. 2018; 59:310-7.
14. Faisal R, Ahmad N, Fahed YS, Chiragh S. Anti-arthritic effect of thymoquinone in comparison with methotrexate on pristane induced arthritis in female Sprague Dawley Rats. Journal of Ayub Medical College Abbottabad. 2018; 30(1):3-7.
15. Roy T, Banerjee I, Ghosh S, Dhali RS, De Pati A, Tripathi S K. Effects of co-treatment with pioglitazone and methotrexate on experimentally induced rheumatoid arthritis in Wistar albino rats. Indian journal of pharmacology. 2017; 49(2): 168.
16. Goncalves GA, Soares AA, Correa RCG, Barros L, Haminiuk CWI, Peralta RM, Ferreira I CFR, Bracht A. Merlot grape pomace hydroalcoholic extract improves the oxidative and inflammatory states of rats with adjuvant-induced arthritis. Journal of Functional Foods. 2017; 33(1): 408-418.
17. Qiao Z, Tang J, Wu W, Tang J, Liu . Acteoside inhibits inflammatory response via JAK/STAT signaling pathway in osteoarthritic rats. BMC complementary and alternative medicine.2019; 19(1): 1-8.
18. Rengasamy KRR, Khan H, Gowrishankar S, Lagoa R JL, Mahomoodally F M, Khan Z, Suroowan S, Tewari D, Zengin G, Hassan STS, Pandian SK. The role of flavonoids in autoimmune diseases: Therapeutic updates. Pharmacology & Therapeutics, 2019; 194: 107-131.
19. Ananth DA, Rameshkumar A, JeyadevI R, Aseervatham GSB, Sripriya J, Bose PC, Sivasudha T. Amelioratory effect of flavonoids rich *Pergularia daemia* extract against CFA induced arthritic rats. Biomedicine & Pharmacotherapy, 2016; 80:244-252.
20. Saleem A, Saleem M, Akhtar MF, Shahzad M, Jahan S. *Moringa rivae* leaf extracts attenuate Complete Freund's adjuvant-induced arthritis in Wistar rats via modulation of inflammatory and oxidative stress biomarkers. Inflammopharmacology, 2020; 28(1): 139-151.
21. Malik K, Ahmad M, Zhang G, Rashid N, Zafar M, Sultana S, et al. Traditional plant based medicines used to treat musculoskeletal disorders in Northern Pakistan. European Journal of Integrative Medicine. 2018; 19:17-64.

The Authors:

Dr. Maheen Fatima
P.G. Trainee,
Department of Pharmacology,
Postgraduate Medical Institute, Lahore.

Dr. Moneeb Ashraf
Associate Professor,
Department of Pharmacology,
King Edward Medical University, Lahore.

Dr. Urooj Fatima
Senior Demonstrator,
Department of Pharmacology,
Fatima Memorial Hospital, Lahore.

Dr. Muhammad Imran
Assistant Professor,
Department of Histopathology,
Allama Iqbal Medical College, Lahore.

Dr. Amer Hassan Siddiqui
Assistant Professor,
Department of Pharmacology,
Postgraduate Medical Institute, Lahore.

Dr. Tafzeel Fatima
House Officer,
Department of Medicine,
Sir Ganga Ram Hospital, Lahore.

Corresponding Author:

Dr. Moneeb Ashraf
Associate Professor,
Department of Pharmacology,
King Edward Medical University, Lahore.
E-mail: moneeb-ashraf@hotmail.com