Proceedings S.Z.M.C. Vol: 36(1): pp. 49-55, 2022. PSZMC-834-36-1-2022

Therapeutic Effect of Berberine Versus Methotrexate on Histopathology in a Rat Model of Pristane-Induced Arthritis

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ABSTRACT

Introduction: Treatment options for rheumatoid arthritis have potentially fatal adverse effects and failure to achieve complete cure. Alternative medicines are, therefore, being researched for this purpose. Berberine is one of such compounds with high antioxidant activity that may prove beneficial in this disease.
Aims & Objectives: To compare effects of berberine with methotrexate on pristane induced arthritis in rats.
Place and duration of study: Post Graduate Medical Institute, Lahore; March to May 2014.
Material & Methods: Forty female Sprague Dawley rats were allotted to five groups including a berberine control. Arthritis developed in 14 days with a single intradermal injection of pristane in arthritis control and experimental groups. Starting Day 15, berberine and methotrexate were administered as single daily intraperitoneal injection for next 14 days. Arthritis resolution was assessed by measuring body weight, clinical score of arthritis on day 0, 14 and 28 and joint histopathology terminally. Data was analyzed using SPSS version 20, p value <0.05 was considered significant.
Results: Arthritis induction reduced body weight in pristane administered groups (142.87 ± 3.56, 146.25 ± 7.49, 112.37 ± 6.23, 114.50 ±3.85, 113.62 ± 7.72 g in Group I, II, III, IV and V respectively) at day 14. Berberine and methotrexate treatment restored body weight in comparison to continuous loss in arthritis control animals on day 28 (155.87 ± 3.72, 162.00 ± 7.96, 105.25 ± 8.04, 133.75 ± 4.89, 133.12 ± 9.24 g in Group I, II, III, IV and V respectively). Berberine and methotrexate both reduced joint inflammation (clinical score 15±1.51, 6.75±1.48, 3.25±1.48 in arthritis control, berberine and methotrexate treated groups). Methotrexate was, however, more effective in reducing clinical arthritis score than berberine on day 28 (p value <0.001). Histopathological changes were reversed similarly by both drugs.
Conclusion: Berberine is effective in treating rheumatoid arthritis though less than methotrexate.

Key words: Rheumatoid arthritis, Joint inflammation, Berberine, Methotrexate

INTRODUCTION

Rheumatoid Arthritis (RA) is a common autoimmune disease of chronic nature that affects many body systems especially synovial tissues, cartilage and bone. It is a highly debilitating disease causing disability, early deaths and is responsible for adverse socioeconomic outcomes.[1]

The major histopathological changes in rheumatoid arthritis include synovial hyperplasia, with inflammatory cells including lymphocytes and macrophages along with fibroblasts, all collectively known as a pannus. The pannus causes destruction of the underlying cartilage and bone by invasion and erosion. The synovial cavity is filled with inflammatory exudate comprising mainly of plasma containing neutrophils.[2]

Upon diagnosis of a patient with RA the main aim of the management is either to achieve complete remission or decrease the disease activity so as to minimize the joint damage, debility and systemic involvement of RA.[3]

Pharmacotherapy options include NSAIDs, corticosteroids and biological & non-biological DMARDs – disease modifying anti-rheumatic drugs. Methotrexate is a widely used first line non-biological DMARD given alone or in combination. It inhibits DNA synthesis and replication by inhibiting dihydrofolate reductase. For the doses used in treatment of RA, it is thought to inhibit enzymes engaged in purine synthesis, leading to adenosine accumulation; thus inhibiting activation of T cells.[4]

It is being used for RA since 1970 and it has proved to be quite effective in providing clinical improvement in at least 50% of RA patients using it. However, it has adverse effects, few of which may be serious, including bone marrow suppression and hepatotoxicity.[5]

Alternative medicines are now also being used to treat various diseases including RA. Among them, medicinal herbs are being used by a large number of people as they are thought to have less adverse effects. Berberine is a major chemical constituent of *Berberis lycium Royle (BLR),* a member of *Berberidiaceae* family of medicinal herbs. BLR, which is widely present in Gilgit, Balistan, Kashmir and Swat has found to have anti-diabetic, anti-hyperlipidemic, hepatoprotective, anti-bacterial, anti-cancer and most important in relevance to RA, anti-inflammatory effects.[6]

Various studies have been carried out to elicit the anti-inflammatory mechanisms of berberine. These include the effects like reduced pro-inflammatory cytokines level including interleukin-6 (IL-6), interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), prostaglandin E2 (PGE_2), nitric oxide (NO) and preventing expression of mRNA for COX-2 (cyclooxygenase-2).[7]

At the molecular level, there is a current and convincing proof for involvement of Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway in pathogenesis and inflammation development related to autoimmune diseases like RA and other autoimmune diseases. Berberine was found to bind to Janus kinase 3 (JAK-3) and inhibits its phosphorylation in an animal model of arthritis.[8]

Therefore, with this scientific basis for the hypothesis that berberine has significant anti-inflammatory activity, present study was carried out for studying histopathological effects in a pristane-induced arthritis rat model and compare them with that of methotrexate, taken as standard treatment.

MATERIAL AND METHODS

This experimental study was performed at PGMI (Post Graduate Medical Institute), Lahore, Pakistan during year 2014 after approval from Ethical Committee of PGMI (No: 8723, Dated: 15-06-2012). Adult female Sprague Dawley, 7-8 week age rats were obtained from University of Veterinary and Animal Sciences. They were kept and acclimatized in animal house at PGMI, Lahore for 7 days. Duration of intervention after acclimatization was 28 days.

Study Design

Animal Grouping: Forty rats weighing between 100-140 grams were randomly divided into five groups labelled from I to V with 8 rats in each group (Table-1).

Group	*Group Name*	*Arthritis induction*	*Treatment Day 15 onwards*
I	Normal Control	No	1 ml/kg normal saline i.p. daily
II	Berberine Control	No	2.5 mg/ml/kg Berberine i.p. daily
III	Arthritis Control	Yes	1 ml/kg normal saline i.p. daily
IV	Berberine Treated Group	Yes	2.5 mg/ml/kg Berberine i.p. daily
V	Methotrexate Treated Group	Yes	0.5 mg/ml/kg methotrexate i.p.daily

i.p. (intraperitoneal)

Table 1: Grouping of rats showing induction of arthritis and experimental interventions (n=8)

Induction of arthritis: Half ml of Pristane (Sigma, USA) was injected intradermally near rat's tail base to animals in arthritis control (group III), berberine treated (group IV) and methotrexate treated (group V) on day 0. Arthritis was induced within 14 days.[9] The progress and severity of arthritis was scored from 0-16 by grossly examining all four limbs of every animal on day 0, 14 and 28.

Administration of berberine and methotrexate: Administration of berberine was started on day 15, after two weeks of arthritis induction by pristane, as a single daily intraperitoneal injection in dose of 2.5 mg/kg to rats in berberine control (group II) and berberine treated (group IV) groups.[10] It was continued for next 14 days. Methotrexate was administered to rats in methotrexate treated group in a similar manner (group V) using 0.5 mg/kg dose.[11] Fresh solution of both drugs was prepared daily.

Parameters

Body weight: Body weight of all animals was measured on day 0, 14 and 28.

Clinical score of arthritis: Joint inflammation in all animals was assessed through clinical score of arthritis. It was calculated for one limb as 0 (no swelling or tenderness at all), 1 (involving one joint), 2 (involving two joints), 3 (involving more than two joints) and 4 (severe arthritis involving entire paw). The result for four limbs of one animal was added to get cumulative score of that animal.[12]

Histopathology: Ankle joints of all rats were amputated after their sacrifice at the end of study. Hematoxylin and eosin-stained slides were prepared after formalin fixation and decalcification by keeping them for 4-5 days in formic acid-formalin solution. Histopathological scoring was done in two paws of each rat separately by studying parameters given in Table-2 and adding them to get cumulative score for that animal.[13]

Parameter	Score	Description
Infiltration of synovium with mononuclear cells	0	No infiltration
	1	Mild
	2	Moderate
	3	Severe
Synovial Cell hyperplasia	0	1 – 3cell layers
	1	4 – 6 cell layers
	2	7 or above cell layers
Villous hyperplasia	0	Absent
	1	Short, few and scattered
	2	Finger like and marked
	3	Diffuse but marked
Pannus Formation	0	Absent
	1	Synoviocyte invasion - mild
	2	Synoviocyte and inflammatory cells- moderate
	3	Synoviocyte and inflammatory cells - Severe

Table-2: Histopathological parameters studied in H&E stained rat ankle slides with their scoring and grading. Each parameter was scored in two paws of an animal to obtain cumulative score for grading.

Statistical analysis:

Data was analyzed using SPSS version 20. Normality was checked by Shapiro Wilk test. Mean±SD, one-way ANOVA and post hoc Tukey and paired t-test were applied to quantitative variables, i.e., body weight and clinical score of arthritis. Frequency percentages were calculated for qualitative data obtained from joint histopathology. Kruskal Wallis ANOVA and Mann Whitney U tests were used to determine overall and group wise differences in histopathology.p value <0.05 was considered significant.

RESULTS

Body Weight (Table-3, Fig-1)

Body weight of normal control (Group I) and berberine control group (Group II) rats persistently increased throughout the study. Arthritis induction in arthritis control (Group III), berberine treated (Group IV) and methotrexate treated (Group V) group significantly reduced their body weight than normal control (Group I) and berberine group (Group V) till day 14 (p value < 0.001). Treatment with berberine and methotrexate in Group IV and V, however, restored their body weight above respective baseline at end of the study. It did not approach the normal control value but on Day 28, it was significantly higher than arthritis control (Group III) (p value <0.001) which showed continuous decline in body weight till end of the study. The difference between berberine (Group IV) and methotrexate (Group V) treated groups themselves was non-significant on Day 28.

Group	Day 0	Day 14	Day 28
Normal Control	119.87 ± 4.54	142.87 ± 3.56$^{\#}$	155.87 ± 3.72$^{\#}$
Berberine Control	120.63 ± 7.32	146.25 ± 7.49$^{\#}$	162.00 ± 7.96$^{\#}$
Arthritis Control	118.50 ± 6.78	112.37 ± 6.23*	105.25 ± 8.04*
Berberine Treated	122.00 ± 5.04	114.50 ±3.85*	133.75 ± 4.89$^{*\#}$
Methotrexate Treated	121.00 ± 7.85	113.62 ± 7.72*	133.12 ± 9.24$^{* \#}$

Table-3: Body weight (g) of pristane induced arthritic rats (n=8) shown as mean±S.D. – Effect of Berberine and methotrexate.

*p value ≤ 0.001 versus normal control

$^{\#}$p value ≤ 0.001 versus arthritis control

Clinical Score of Arthritis (Table-4)

No inflammation (clinical score 0) developed in normal control (Group I) and berberine control group (Group II) throughout the study. Arthritis was induced in arthritis control (Group III) and experimental groups (Groups IV and V) with almost similar clinical score on Day 14. Berberine and methotrexate treatment after Day 14 reduced the inflammation and its clinical score in berberine (Group IV) and methotrexate treated (Group V) groups respectively on Day 28. The score in arthritis control group (Group III), in comparison, remained unchanged after induction of arthritis between Day 14 and 28. Though berberine markedly reduced the inflammation of joints in Group IV than arthritis control (Group III) as measured on Day 28 (p value < 0.001), it was still significantly higher (p value < 0.001) than methotrexate treated group (Group V) which had the least value of clinical score on Day 28.

Group	Day 0	Day 14	Day 28
Normal Control	0	0	0
Berberine Control	0	0	0
Arthritis Control	0	15 ± 1.51	15 ± 1.51$^{\#}$
Berberine Treated	0	14.50 ± 1.77	6.75 ± 1.48$^{* \#}$
Methotrexate Treated	0	15.25 ± 1.48	3.25 ± 1.48*

Table-4: Clinical score of inflammation of pristane induced arthritic rats (n=8) shown as mean ± S.D. – Effect of Berberine and methotrexate.

$^{*}p$ value ≤ 0.001 versus arthritis control

$^{\#}p$ value ≤ 0.001 versus methotrexate treated group

Joint Histopathology (Table-5 & Fig-2*a-e*)

All parameters were normal in normal control (Group I) and berberine control group (Group II) while various grades of these derangements were present in all animals of arthritis control (Group III). Berberine and methotrexate treatment reversed these pathologies to normal in some animals and reduced

their overall severity in others in Group IV and V respectively (Fig 3*a-e*). The statistical difference for these groups versus normal control (Group I) and berberine control (Group II) groups, however, remained significant for most of the parameters.

Inflammation by mononuclear cells was significantly reduced than arthritis control (group III) in groups treated with berberine (p value < 0.001) and methotrexate (p value 0.001). This decrease was more significant in methotrexate treated group (Group V) than berberine treated group (Group IV) (p value 0.013) approaching a non-significant difference as compared with normal control (group I) having p value of 0.143.

Synovial hyperplasia was less affected by berberine treatment (p value 0.175 vs arthritis control, Group III) as compared to methotrexate which caused significant amelioration as compared to arthritis control (Group III) (p value 0.018). The difference between berberine and methotrexate treatment themselves was, however, non-significant.

Villous hyperplasia and pannus formation were also significantly less in both berberine treated (Group IV) and methotrexate treated (Group V) groups than arthritis control (Group III) (p values 0.007 and 0.019 for villous hyperplasia and 0.01 and 0.05 for pannus formation respectively). Severity of these two changes was, however, less in berberine treated group than methotrexate treated group, but the difference was statistically non-significant.

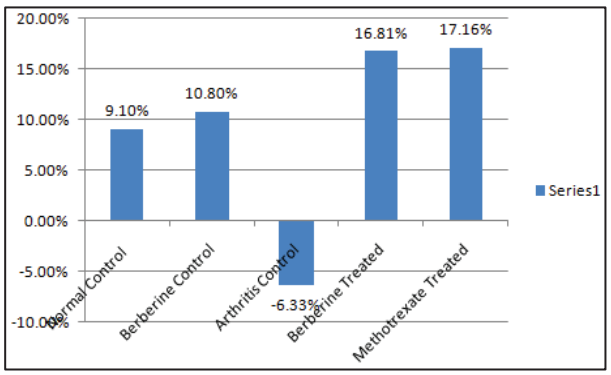

Fig-1: Body weight change (%) from day 14 to 28 in all study groups.

Parameter	Grading	Normal Control	Berberine Control	Arthritis Control	Berberine Treated	Methotrexate Treated
Infiltration of synovium with mononuclear cells	Normal	*100*	*100*	*0*	*12.5*	*75*
	Mild	*0*	*0*	*12.5*	*75*	*25*
	Moderate	*0*	*0*	*62.5*	*12.5*	*0*
	Severe	*0*	*0*	*25*	*0*	*0*
	p value	###	###	***	*** ### ^	###
Synovial Cell hyperplasia	Normal	*100*	*100*	*0*	*12.5*	*37.5*
	Mild	*0*	*0*	*62.5*	*75*	*62.5*
	Moderate	*0*	*0*	*37.5*	*12.5*	*0*
	Severe	*0*	*0*	*0*	*0*	*0*
	p value	###	###	***	***	** #
Villous hyperplasia	Normal	*100*	*100*	*0*	*25*	*37.5*
	Mild	*0*	*0*	*37.5*	*75*	*50*
	Moderate	*0*	*0*	*50*	*0*	*12.5*
	Severe	*0*	*0*	*12.5*	*0*	*0*
	p value	###	###	***	** ##	** #
Pannus Formation	Normal	*100*	*100*	*0*	*12.5*	*12.5*
	Mild	*0*	*0*	*12.5*	*62.5*	*37.5*
	Moderate	*0*	*0*	*62.5*	*25*	*50*
	Severe	*0*	*0*	*25*	*0*	*0*
	p value	###	###	***	*** #	*** #

Table-5: Percentages of four histological criteria in various groups of rats with pristane induced arthritis (n=8) and their statistical significances – Effect of Berberine and methotrexate.

$^{***}p$ value ≤ 0.001, $^{**}p$ value ≤ 0.01 versus normal control

$^{###}p$ value ≤ 0.001, $^{##}p$ value ≤ 0.01, $^{#}p$ value ≤ 0.05, $^{###}$versus arthritis control

$^{\wedge}p$ value ≤ 0.05 versus methotrexate treated group

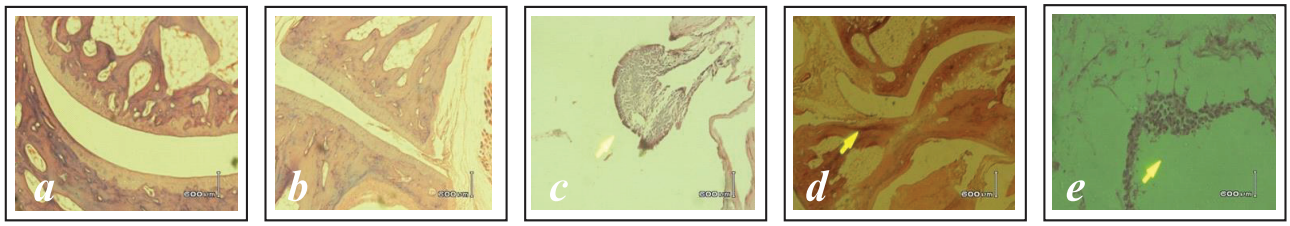

Fig-2a-e: Histopathological Findings

- **a.** Normal synovial joint anatomy in normal control group
- **b.** Normal synovial joint histology in berberine treated group
- **c.** Villous hyperplasia in arthritis control group
- **d.** Pannus formation in berberine treated group
- **e.** Synovial hyperplasia in methotrexate treated group

DISCUSSION

This study was designed to evaluate berberine's anti-inflammatory effect in the rat model, coupled with any physiological changes such as body weight in comparison with methotrexate .

Use of pristane is one of the several techniques available to induce arthritis in animal models.[14] Polyarthritis develops in about 14 days after a single intradermal injection of pristane and closely resembles in its clinical and histopathological findings to RA.

Arthritis induction decreased body weight of all animals in disease control, berberine and methotrexate groups while animals in normal control and berberine alone group got a steady and almost similar increase in body weight throughout the study. Other works have also reported decrease in body weight after arthritis induction with pristane.[15] Methotrexate and berberine both restored body weight in respective groups above baseline in comparison to disease control which lost weight continuously till the end of study. The percentage of restoration in weight (between day 14 and 28) was similar in both groups and was more than both control groups. This improvement in weight was most probably due to decrease in arthritis and not the effect of methotrexate or berberine themselves because methotrexate itself has a negative effect on weight in healthy animals[16] and animals in berberine control group of this study did not show any significant gain in body weight over normal control till the end of study. This finding is also supported by works done on methotrexate[17] and berberine[18] where resolution of arthritis has resulted in improvement of body weight.

Clinical score of arthritis was similar in arthritis induced groups on day 14. This was in accordance with previous works done with pristane,[14] signifying a successful induction of disease model in this study. Administration of methotrexate and berberine both reduced the score significantly in their respective groups as compared to disease control in which the scored remained high till end of the study. Methotrexate was, however, more effective than berberine as difference between these two was also significant on day 28. Experiments using berberine in other models of RA also affirm these results. Wang et. al., 2014 demonstrated improvement in clinical arthritis score by using berberine in a rat model of collagen induced arthritis.[18] Kim et. al., 2011 reported dose dependent decline in joint oedema by 25% and 47% with berberine as compared to placebo in carrageenan/kaolin induced mono arthritis in rats.[19]A relatively recent study demonstrated prevention of paw edema when berberine was administered to Freund's adjuvant induced arthritic Sprague Dawley rats from first day of induction.[20]

H & E stained slides of ankle joints were studied for infiltration by inflammatory cell, synovial hyperplasia, pannus formation and villous hyperplasia. While these parameters were deranged in all animals of disease control group, severity and percentage of animals developing these changes were significantly less than arthritis control in methotrexate and berberine treated groups. Methotrexate and berberine were almost equally effective in preventing these changes except for infiltration of inflammatory cells which was significantly less in methotrexate than berberine treated group. Reduction in these histopathological changes was also demonstrated in previous works on berberine incollagen-induced arthritis,[18] Carrageenan/kaolin-induced kneemonoarthritis[19] and adjuvant-induced rheumatoid arthritis.[21] All these studies used Sprague Dawley rats, the strain used in our study. Berberine not only ameliorated joint destruction in rats but also in arthritic mice.[22]

One facet in pathogenesis of RA is increased oxidative stress.[23] Different antioxidants have, therefore, been employed in an attempt to seek some effective treatment with less adverse effects.[24] Berberine is an alkaloid with strong antioxidant with

consequent anti-inflammatory properties, with multiple mechanisms underlying these anti-inflammatory actions being postulated.[25] Dinesh and Rasool, 2019 demonstrated in a rat model that berberine effectively reduces proliferation of Th17 cells and responses of synoviocytes to IL-21 thus effectively preventing inflammation in RA.[26] The resolution of arthritis obtained in this study may also be due to these anti-inflammatory properties of berberine.

This study used standard methodology for induction of arthritis and evaluation of clinical inflammation as well as joint histology but limited by absence of advanced parameters of underlying mechanism.

CONCLUSION

Berberine is effective in treating RA though less as compared to methotrexate. As doses of methotrexate used to treat RA carry a risk of fatal adverse effects and methotrexate or berberine alone do not confer complete amelioration of RA, combining berberine with methotrexate may enhance efficacy of treatment and reduce the dosage of methotrexate, too, resulting in fewer adversities. It may be used in combination with methotrexate to enhance efficacy and reduce required dose of methotrexate.

Acknowledgement:
This project was funded by PGMI, Lahore. Thanks are due to animal house staff and histopathology colleagues at PGMI for their untiring help.

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