Prolotherapy Improves Anatomical and Clinical Outcomes in Monosodium Iodoacetate Induced Model of Osteoarthritis

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ABSTRACT

Objective: To assess the ameliorative effects of Prolotherapy on the pain score of the gait cycle in Monosodium Iodoacetate (MIA) induced osteoarthritis in the rat knee joint.

Study Design: Laboratory-based experimental study.

Place and Duration of Study: Department of Anatomy, Army Medical College, Rawalpindi in collaboration with the National Institute of Health (NIH), Islamabad and Pathology Lab Pak Emirates Rawalpindi, Pakistan, from Aug to Nov 2021.

Methodology: Thirty male Sprague Dawley rats were randomly divided into three groups (n=10 in each group). The control group was Group-A. To induce osteoarthritic changes in Group-B, a single dose of 1mg Monosodium Iodoacetate was injected intra-articularly into the right knee. Group-C received a single dose of 1mg Monosodium Iodoacetate injection in the right knee intra articularly to induce osteoarthritic changes, and was followed by 0.1ml Prolotherapy (3ml of 25% dextrose, 2ml of 2% Xylocaine, 1ml of injection thiamine, and 1ml of injection Methylcobalamin) at 2, 6 and 10 weeks intra articularly into the right knee joint. Before euthanasia, Pain scoring schemes for gait in rats of all groups were done at 0, 2 and 14 weeks.

Results: The present study concluded that prolotherapy improved the gait cycle pain score in experimental group C (*p*-value 0.001 on the intergroup comparison at two weeks and 14 weeks while *p*-value 0.001 when compared pain score within the Group C at 0, 2 and 14 weeks).

Conclusion: Prolotherapy had ameliorative effects on the pain score of the gait cycle in experimental Group C on Monosodium Iodoacetate induced osteoarthritic changes in the knee joint of a rat.

Keywords: Gait cycle, Monosodium iodoacetate, Osteoarthritis, Prolotherapy.

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INTRODUCTION

Osteoarthritis (OA) is arthritis that mostly affects weight-bearing joints. Knee, hip, and vertebral joints are commonly affected.[1] The articular cartilage, meniscus, subchondral bone, ligaments, and surrounding structure of the joint synovial membrane and infrapatellar fat pad) are affected by OA.[2] Currently, no treatment options are available to reverse the debilitating effects of osteoarthritis.[3] Oral and intra-articular medications are currently focused on reducing inflammation and relieving pain, with surgical arthroplasty as a last resort.[4] Regenerative medicines have revolutionized the treatment of knee osteoarthritis in recent years. These products help the joint remodel and heal on its own, resulting in less discomfort and more mobility and potentially eliminating the need for invasive surgery.[5] Animal models are the most effective alternative to study human OA, allowing for better diagnosis and following disease progression.[6] intraarticular Injection of Monosodium Iodoacetate (MIA) is commonly used to induce osteoarthritic changes, and the resulting changes are identical to human degenerative OA.[7] MIA causes cellular death in chondrocytes by inhibiting aerobic glycolytic pathways, and this results in the reduction of chondrocyte density which subsequently produces morphological and microscopic pathologies in joints.[8]

Chronic diseases of the musculoskeletal system, including knee osteoarthritis, are treated with injectable prolotherapy.[9] Intraarticular prolotherapy injections stimulate the release of growth factors that helps injured cartilage and soft tissue proliferate and regenerate.[10] Prolotherapy has drawn our attention for being a simple, inexpensive procedure with a high safety margin and can easily be administrated in a primary care setting.

This study aimed to determine the ameliorative effects of prolotherapy on the pain score of the gait cycle in MIA-induced osteoarthritis in the rat knee joint.

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METHODOLOGY

This study was conducted at the Department of Anatomy, Army Medical College/National University of Medical Sciences Rawalpindi Pakistan in collaboration with the National Institute of Health (NIH), Islamabad and Pathology Lab Pak Emirates Rawalpindi Pakistan, from August to November 2021. All animal care and handling procedures were performed after getting approval from the ethics committee of Army Medical College/National University of Medical Sciences, Rawalpindi (IERB number 330).

Thirty male Sprague Dawley rats were obtained from NIH, Islamabad. Rats were kept at the animal house of NIH under standard conditions and were allowed free access to a standard lab diet and clean drinking water.

Inclusion criteria: Male rats of age 3-4 months, and weight 250+50 grams were included in the study.

Exclusion criteria: Rats having any gross joint deformity were excluded from the study.

Rats were randomly divided into three groups, each having ten rats with five rats per cage. In Group-A, no intervention was done in this control group, Group-B, was injected intra articularly with a single dose of 1mg Monosodium Iodoacetate in the right knee to induce osteoarthritic changes, and group C, injected intra articularly with a single dose of 1mg Monosodium Iodoacetate in the right knee to induce osteoarthritic changes and was followed by 0.1ml intra-articular injection of prolotherapy at week 2, 6 and 10 in the right knee.

Pain scoring schemes for gait in rats of all groups were done at week 0 (baseline), week 2 (recommended duration of osteoarthritis induction), and week 14 (one month after the last dosage of prolotherapy) before euthanasia.[11-13] A comparison of the pain scoring scheme of gait was made between and within the groups. For gait analysis, the ventral surface of each rat's rear feet was inked with black ink.

Rats were prompted by food to walk the entire length of A4 paper. The osteoarthritic leg's footprints were compared to the left leg's to assess weight-bearing during movement. Gait was scored as shown in Table-I.

IBM SPSS (Statistical Package for the social sciences) version 22 was used to analyzed the data. Descriptive statistics, i.e., Mean ± SD, was used to describe the quantitative variables. Next, a one-way analysis of variance (ANOVA) was applied for intergroup comparison, followed by Post- Hoc Tukey's test; if the *p*-value ≤0.05, the difference between the two observations was considered significant.

Table-I: Pain scoring scheme for gait in rats.

Gait Scores	Description/Criteria
0	Normal, equal ink staining on both feet
1	A slight limp, toe staining evident, and some heel staining for all steps, no carrying or dragging.
2	Limping toes only staining for all steps, no carrying or dragging.
3	Dragging and carrying leg, black drag marks from the dorsal side of foot present or some attempt to use right as evidenced by minimal toe staining in at least one print
4	Carrying leg entire time, no staining from the painful leg or only minor black drag marks[13]

RESULTS

Thirty male Sprague Dawley rats, of age 3-4 months, and weight 250 ± 50 grams, were included. There was a statistically significant difference in the pain scoring of Group-B and Group-C (p<0.001), Gait score was 0 in all rats of each group at 0 weeks, while the Gait score was 1.6 ± 0.516 and 1.6 ± 0.516 in experimental groups B and C respectively at two weeks. Gait score was 2.9 ± 0.875 and 0.3 ± 0.483 in experimental groups B and C, respectively, at 14 weeks, shown in Table-II.

Table–II: Comparison of mean values of gait score (at 2 and 14 weeks) between control Group-A, experimental Group-B, and experimental Group-C.

Assessment of Pain (Gait Score)	Group-A (n= 10)	Group–B (n= 10)	Group–C (n= 10)	*p*-value
at 2 weeks	Nil	1.600 ± 0.516	1.600 ± 0.516	<0.001
at 14weeks	Nil	2.900 ± 0.875	0.300 ± 0.483	<0.001

There was no statistically significant difference between Group-B and C (p-value=1.000) at two weeks. Group-A and C had no statistically significant difference (p=0.486) at 14 weeks, as shown in Table-III**.**

Table-III: inter Group comparison of mean values of gait score (at 2 and 14 weeks) between control Group-A, experimental Group-B, and experimental Group-C.

Gait Score	A vs B	A vs C	B vs C
at 2 weeks	0.001	0.001	1.000
at 14weeks	0.001	0.486	0.001

In experimental group B, the Gait score was zero (0) at 0 weeks. At the same time, Gait score was 1.600 ± 0.516 and 2.900 ± 0.875 at two weeks and 14 weeks, respectively (p<0.001), While Gait score was 1.600 ± 0.516 and 0.300 ± 0.483 at two weeks and 14 weeks respectively in Group-C (p<0.001) (Table–IV). Table-V

showed an intergroup comparison of groups at 0, 2 and 14 weeks.

Table-IV: Comparison of mean values of control group A, experimental group B and C at gait score at 0, 2, 14 weeks.

Groups	At 0 week	At 2 week	At 14 Week	*p*-value
Group-B (n=10)	Nil	1.600 ± 0.516	2.900 ± 0.875	<0.001
Group-C (n=10)	Nil	1.600 ± 0.516	0.300 ± 0.483	<0.001

Table-IV: Intergroup comparison of pain score at 0, 2 and 14 week.

Groups	Week vs 2 Week	2 Week vs 14 Week	Week vs 14 Week
Group–B, (n= 10)	0.001	0.001	0.001
Group–C, (n= 10)	0.001	0.486	0.245

DISCUSSION

Regarding the gait analysis, while comparing within and between the groups, rats in control Group-A showed equal ink staining on both feet, reflecting normal gait. Results were supported by the study conducted by Sadek *et al*, who reported that there were no signs of pain behaviour and gait abnormality in any group before MIA injection.[12] Rats in experimental Group-B showed highly significant results with highly significant deterioration of gait reflected by limping toes and dragging leg when gait score was compared within the group. with control Group-A and experimental Group-C. These results were supported by the same study conducted by Sadek *et al*, who found that the MIA model of OA in rats produces pain behaviour similar to that exhibited in OA patients, as well as abnormal weight-bearing and gait abnormalities. MIA causes chondrocytes degeneration, necrosis, and articular cartilage degradation as early as the first day after intraarticular injection.[14] That explains the early pain behaviour in this group.[12] Miyagi *et al*, reported that the MIA injection model appears preferable for assessing gait parameter and pain behaviour as compared to surgically induced, as the MIA model cause significant joint damage.[15]

On the other hand, rats in experimental Group-C showed significant improvement in gait score in the form of improved ink staining of both limbs and slight limp when gait score was compared within the group and with control Group-A and experimental Group-B. This improvement was due to relief in pain after prolotherapy. Our results were in line with the study conducted by Korntners *et al.* This study was conducted on female Lewis rats with Achilles tendon injury.[16] A study showed that by stimulating the release of growth factors, Intra-articular prolotherapy injection aids in cartilage and soft tissue proliferation and regeneration, which results in a considerable reduction of pain and disease progression.[17] Prolotherapy induces a pro-inflammatory response that results in the release of growth factors and cytokines, ultimately resulting in a regenerative process within the affected joint and opening potassium channels by hyperpolarizing nociceptive pain fibres, resulting in reduced pain perception.[11] Findings in the present study were paralleled with the study conducted by Rezasoltani *et al,* in which pain score and disability score were improved after prolotherapy injection.[18] A study conducted by Sit *et al*, showed that patients suffering from knee osteoarthritis had improved function and quality of life after intra-articular injection of prolotherapy by reducing pain, which is similar to the present study in the form of marked improvement in gait score of rats in an experimental Group-C.[10] Jensen *et al*, found similar results in their study in which there was a significant improvement in pain and gait after prolotherapy injection in rats with Achilles tendinopathies.[19]

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CONCLUSION

The present study concluded that prolotherapy ameliorated the pain scoring scheme of the gait cycle on MIA-induced osteoarthritis in the rat knee joint.

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Conflict of Interest: None.

Author's Contribution

AZ:, KQ: Direct and intellectual contribution to the title, design, data collection, analysis, interpretation, results and conclusion, AQ:, ZI: Intellectual contribution to design, data analysis, and interpretation of results, TF: Intellectual contribution to data analysis, and interpretation conclusion, SS: Intellectual contribution to data analysis, and interpretation.

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