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Comparative Effect of Antidepressants (Duloxetine) and NSAID (Dexibuprofen) in a New Rat Model of Chronic Pain Induced Depression Associated with Monosodium Iodo Acetate (MIA) Induced Osteoarthritis in Rats

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Authors' contributions

This work was carried out in collaboration between two authors. Author VK designed the study, conducted the study, and wrote the first draft of the manuscript. The complete study was done under guidance of author VSS, including manuscript preparation.

Research Article

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ABSTRACT

Objective: To evaluate the role of anti-depressants (Duloxetine) and NSAID (Dexibuprofen) in a new rat model of chronic induced depression.
Methods: Twenty four male wistar rats were divided into 4 groups of 6 animals each. Group I to IV served as vehicle control, osteoarthritis (OA) control, duloxetine and dexibuprofen treated groups respectively. Group I received intra-articular Injection of 50 µl of 0.9% normal saline, and Group II to IV received 50 µl MIA, and the treatment of drugs started on the same day. The animals will be monitored for OA parameters and/or depression on pre-dose day (day 0) and on day 1, 3, 5, 7, 11, 14, 18, 21 and 28 th day.
Results and Discussion: In MIA treated group, the rise in knee inflammation is maximum on day 3 (12.31±0.85 mm; $p<0.001$) and reduced near to normal on day 7 (9.26±0.57 mm; $p<0.001$). Dexibuprofen and duloxetine decreased the inflammation from day 3, and the decrease is comparatively better in dexibuprofen group. Also, dexibuprofen increased vocalization threshold of knee compression force for 7 days and decreased thereafter, whereas duloxetine has no effect for first 7 days and increased thereafter. Duloxetine was significantly ($p<0.001$) effective on neuropathic pain (Punctate allodynia, mechanical

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gripstrength, threshold angle of knee extension) and depression (forced swim test and locomotor activity) compared to dexibuprofen.
Conclusion: The present study has shown that dexibuprofen has the potential in the initial phase of chronic OA and duloxetine in the later stage, where neuropathic and depressive component dominates.

Keywords: Depression; Dexibuprofen; Duloxetine; MIA; Osteoarthritis.

1. INTRODUCTION

Among older adults, osteoarthritis (OA) is one of the most common comorbidities associated with poor sleep affecting 50% of the persons age 65 or older [1]. It is well established that pain interferes with sleep and in turn that disturbed sleep reduces pain threshold [2-4]. Though, depression is significant among patients with arthritis and musculoskeletal illnesses, the impact of depression on osteoarthritis have not been extensively studied. Hence, there are no convincing evidences of therapeutic management concentrating on both chronic pain and its associated depression. Thus there is a need for more studies to be conducted on the treatment regimen of OA associated pain and depression.

The common treatment for OA includes nonsteroidal anti inflammatory drug (NSAID). The role of analgesics in reducing pain induced depression is not studied. Similarly, scientific literatures support the use of antidepressants in rheumatic painful conditions [5-8], but its dual effect on pain and pain induced depression is not studied. The present study was undertaken to determine the pharmacological effect of dexibuprofen and duloxetine in the rat model of Monosodium iodoacetate (MIA) induced OA to address both chronic pain and its associated depression in a single animal model. Dexibuprofen, a NSAID is a well established drug in treating rheumatic pain and inflammation [9]. Duloxetine is the tricyclic antidepressant (TCA) that exerts antinociception effect [10-12].

2. MATERIALS AND METHODS

2.1 Experimental Animals and Induction of Arthritis

2.1.1 Animals

Healthy male wistar rats weighing about 150 – 200 gram (g) were obtained from Venus Medicine Research Centre. The protocol was approved by the institute's animal ethical committee [Approval No: 126/bc/09/ CPCSEA (Committee for the Purpose of Control and Supervision on Experiments on Animals)].

2.1.2 Experimental design

Twenty four male wistar rats were used in this study. The animals were randomized into 4 groups of 6 animals as per body weight.

Group 1: Vehicle control (50 micro-litre (µl) of 0.9% normal saline, Intra-articular injection)
Group 2: Osteoarthritis control (50µl of MIA, Intra-articular injection)
Group 3: MIA (50µl) + Duloxetine [3 milligram (mg)/kilogram (kg)], once daily)
Group 4: MIA (50µl) + Dexibuprofen (30 mg/kg, thrice daily)

The oral drug treatment is started on day 1 and continued upto day 28. Duloxetine and dexibuprofen tablets were dissolved in water for injection before administration.

2.1.3 Induction of osteoarthritis

After appropriate anaesthesia (ketamine 50mg/kg, intra-peritoneal) each rat was positioned on its back and the right leg was flexed 90 degrees at the knee. Test group animals are treated with MIA (4 mg, 50 µl of 80mg/ml MIA dissolved in 0.9% sterile normal saline) by a single intra-articular injection through the intrapatella ligament of the right knee by using unit syringe fitted with 26G, 0.5 inch needle. Control group animals are treated with single intra-articular injection of MIA dose equivalent 0.9% sterile normal saline into the right knee. Treatment will be given to the MIA treated animals from 1st day to 28th day following OA induction [13-15]. The animals will be monitored for induction of OA parameters on pre-dose day (day 0) and on day 1, 3, 5, 7, 11, 14, 18, 21 and 28 th day and for depression on day 9, 18 and 27.

2.2 Osteoarthritis Activity

2.2.1. Measurement of Knee joint diameter

The femorotibial joint diameter of right hind leg for both control (0.9% saline) and MIA (50µl) injected group animals were measured by using calibrated digital caliper. The unit of joint diameter was expressed as millimetre (mm) [16-17].

2.2.2 Evaluation of Mechanical Hyperalgesia

The vocalisation threshold of knee compression was measured with the help of forceps fitted with pressure gauge having the length of 20 cm long and 4mm X 4mm contact area. The pressure was applied continuously over the knee joint until an audible squeak was elicited. The output voltage was calibrated as grams of force [17].

2.2.3 Measurement of Punctate allodynia

Punctate allodynia was assessed based on the withdrawal thresholds to calibrated von Frey hairs. A maximal cut off of 15 g was used. Animals were placed in Plexiglas boxes with a mesh flooring giving access to the underside of their paws and allowed to acclimatize for at least 30 minutes (min). Allodynia was evaluated by application of von Frey hairs in ascending order of force for up to 6 seconds (sec) to the plantar surface of hind paws. The lowest amount of force required to elicit a response was recorded as paw withdrawal threshold (PWT) in g [16, 18-20].

2.2.4 Assessment of Grip strength

Rota rod instrument was used to assess grip strength and muscle co-ordination. The duration of animal grasping the revolving rod (10 cm diameter; 16 rounds per minute (rpm), 20 rpm) was recorded either by manually or automatically depending upon the instrument. The cut off time for this test is 180 sec. During the time of riding, if the animal doesn't fall within 180 seconds the animal was released from the revolving rod [18-21].

2.2.5 Weight bearing

An incapacitance tester (Linton Instrumentation, Norfolk, UK) was employed for determination of hind paw weight distribution. Rats were placed in an angled plexiglass chamber positioned so that each hind paw rested on a separate force plate The force exerted by each hind limb (measured in grams) is averaged over a 5 sec period. Each data point is the mean of three, 5 sec readings. The change in hind paw weight distribution was calculated by determining the difference in the amount of weight (g) between the left and right limbs. Results are presented as the difference in weight bearing between the left (contralateral control) limb and right (osteoarthritic) limb [15].

2.2.6 Struggle threshold angle of knee extension

The study was done as described by Yu YC et al, 2002. The rat was gently restrained by one hand to measure the struggle threshold of knee extension. While holding the rat in the palm, the leg was extended to determine the knee extension angle at which the rat showed struggling behaviour. The extension angle was then calculated by trigonometric function using the length of the tibia and the foot travel distance during extension [17].

2.3 Depressant Activity

Depressant and psychological activity is assessed by Forced swim test and Actophotometer.

2.3.1 Forced swim test

The Forced swim test (FST) was carried out according to Porsolt et al. During the test session, the climbing behaviour, swimming behaviour and immobility time were recorded. Climbing behaviour is defined as upward directed movements of the forepaws along the side of the swim chamber. Swimming behaviour is defined as movement throughout the swim chamber, which include crossing into another quadrant. The rat was judged immobile if it is floated in the water in an upright position and makes only little movements to keep its head above the water or made other passive movements [18-20].

2.3.2 Spontaneous locomotor activity

To evaluate spontaneous locomotor activity, each animal was individually placed in an actophotometer. The photocells of the actophotometer were checked before use and the animals were individually placed in a square arena (30 x 30 cm). After an initial accustomed period (2 min), the locomotor scores were recorded digitally for the next 10 min [19-20].

2.4 Measurement of Body Weight

Body weights of each group animals were measured at alternative days by using weighing balance, and the changes were recorded.

2.5 Statistical Analysis

The data are expressed as the mean ± standard error of mean (SEM). Statistical analyses were conducted by two way analysis of variance (ANOVA), followed by Bonferroni post test

to compare means among groups at every time points. A *P*-value of less than 0.05 was considered to be significant.

3. RESULTS AND DISCUSSION

After 3 days post injection of MIA (Group II; Fig. 1.), brief period of inflammation was noted which reduced to normal levels by day seven as previously reported by Fernihough J et al. 2004 [16]. It correlates with early synovial inflammation that predicts development of OA in the knee [24].

Dexibuprofen (8.61 ± 0.46; p<0.001) and duloxetine (9.15± 0.54) reduced the inflammation on day 7, compared to MIA treated group. The reduction is more in dexibuprofen group compared to duloxetine. The inflammation has slightly increased in duloxetine group from day 7 to day 14, whereas the fluctuation is not seen in dexibuprofen group. This shows that dexibuprofen has major role in reducing OA induced inflammation than duloxetine. As, OA related pain has been attributed to local tissue injury causing nociceptive, neuropathic pain and depression [22-23], the positive effect of dexibuprofen and negative effect of duloxetine before 7 days indicates the importance of dexibuprofen on inflammatory pain induced by OA.

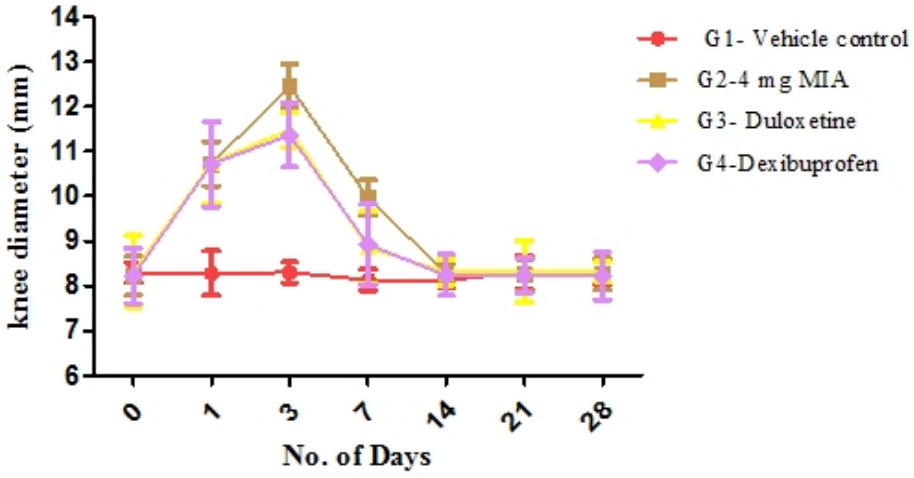

Fig. 1. Comparative effect of Duloxetine and Dexibuprofen on knee joint swelling in MIA induced OA and depression in rats.

Dexibuprofen has significantly (115.00±0.82; p<0.001; Fig. 2.) increased Vocalization threshold of knee compression force on day 3 and had no effect thereafter, whereas duloxetine significantly increased Vocalization threshold from day 7 (94.83 ±0.60; p<0.001) to day 28 (197 ±1.15; p<0.001).

After day 7 following iodoacetate induced OA, behaviour was characterised by profound referred hyperalgesia, allodynia, altered muscle co-ordination, changes in hind paw weight distribution, and change in knee extension angle threshold. OA patients also show hyperalgesic responses to noxious stimuli applied around an osteoarthritic knee [25, 26] and commonly report referred allodynia in other areas adjacent to the affected joint. In animals,

the mechanism by which referred pain is generated from the knee has been studied in inflammatory arthritis.

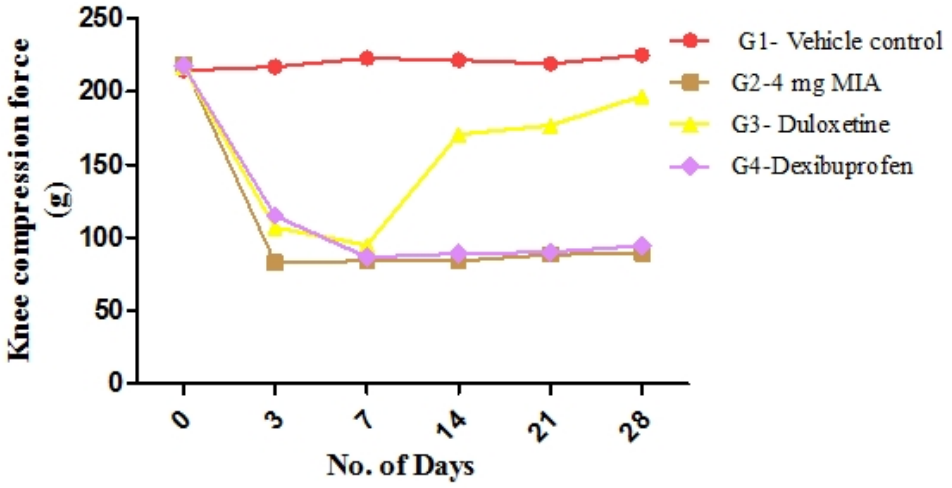

Fig. 2. Comparative effect of Duloxetine and Dexibuprofen on vocalization threshold of knee compression force (Mechanical Hyperalgesia) in MIA induced OA and depression in rats

It is characterised by an increase in afferent activity, lowered threshold of spinal nerves to innocuous stimulation and an increase in the receptive field of spinal neurones to include anatomical regions adjacent to that affected by the initial tissue damage [27]. The clear mechanisms behind the central sensitisation responsible for the generation of pain responses in human OA are currently unknown. Back labelling from the joint space in this study confirmed that the nerves supplying the knee have their cell bodies distributed in dorsal root ganglion (DRGs) in segments between L2–6.These five DRGs form the dorsal root origins for the saphenous (L2–3) and sciatic (L4–6) nerves that innervate the whole of the lower rat limb [28]. Therefore, cell bodies for the nerves from the knee co-localise in DRGs containing cell bodies from nerves supplying the paw. This would allow cross talk at the level of the spinal cord to generate the referred pain measured in the hyperalgesia and allodynia tests. This suggests that the development of OA in the knee may cause the same increase in receptive field size or recruitment of central neurones that occurs as a result of inflammation in the knee [27].

Duloxetine has significantly ($p<0.001$; Fig. 3.) increased paw withdrawal threshold from day 7 (5.5±0.19 ; $p<0.001$) to day 28 (11.7± 0.83 ; $p<0.001$) , increased mechanical grip strength from day 7 (22.00 ±1.18; $P <0.05$; Fig. 4.) to day 28 (29.00±1.06; $p<0.001$), enhanced weight bearing from day 7 (30.67±1.20; $p<0.001$; Fig. 5.) to day 28 (15.00±1.24; $p<0.001$), and enhanced threshold angle of the knee extension from day 3 (21.50 ±1.67; $p<0.001$; Fig. 6.) to day 28 (22.00 ±1.34; $p<0.001$), whereas dexibuprofen has no effect. Duloxetine significantly ($p<0.001$; Fig. 9.) improved the body weight compared to dexibuprofen.

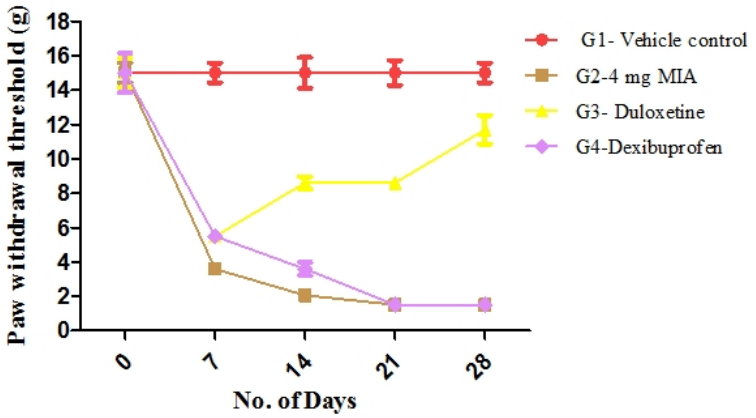

Fig. 3. Comparative effect of Duloxetine and Dexibuprofen on paw withdrawal threshold to von Frey filament stimulation in MIA induced OA and depression in rats

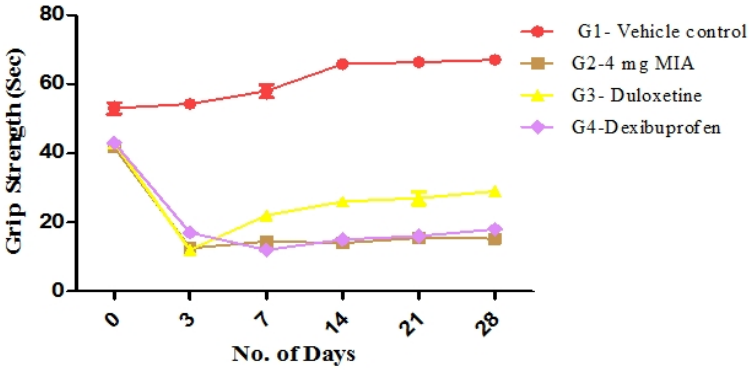

Fig. 4. Comparative effect of Duloxetine and Dexibuprofen on muscle coordination in MIA induced OA and depression in rats

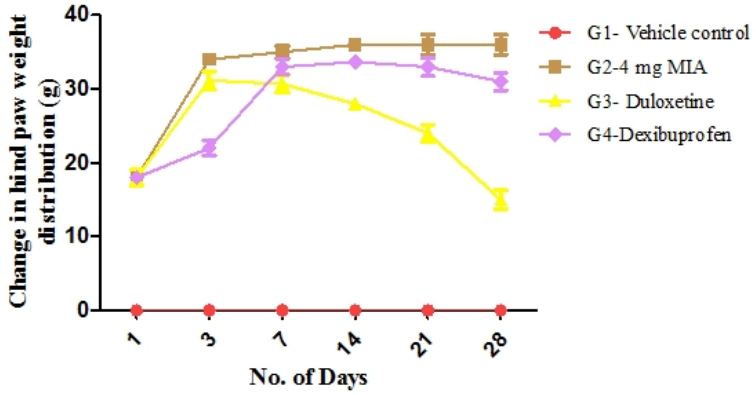

Fig. 5. Comparative effect of Duloxetine and Dexibuprofen on weight bearing in MIA induced OA and depression in rats

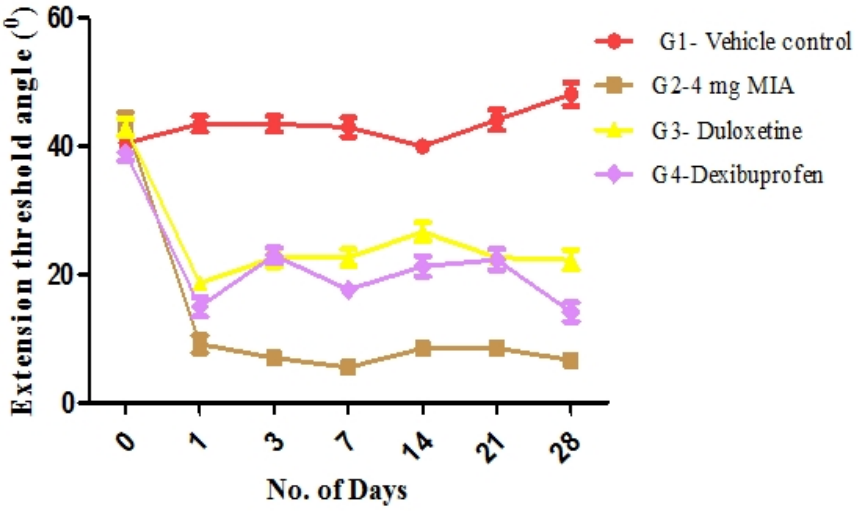

Fig. 6. Comparative effect of Duloxetine and Dexibuprofen on threshold angle of the knee extension in MIA induced OA and depression in rats

Figs. 7 & 8. shows the results of locomotor activity and forced swim test. MIA induced animals (Group-I) has shown significant ($p<0.001$; Fig. 7 & 8.) depression from day 9 and increased till day 28. For the first time, this new animal model has been reported that MIA induced OA can induce depression at defined period. Currently, there is no reported single animal model that can be used to investigate both chronic pain and pain induced depression. The data of this study support the use of MIA induced rat knee OA model as a single animal model to analyse the effect of drugs on chronic pain and pain induced depression.

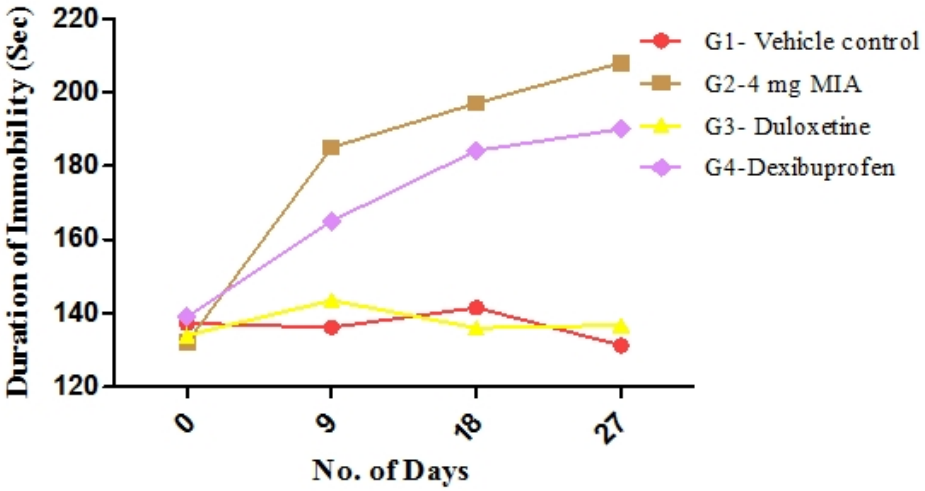

Fig. 7. Comparative effect of Duloxetine and Dexibuprofen on forced swim test in MIA induced OA and depression in rats

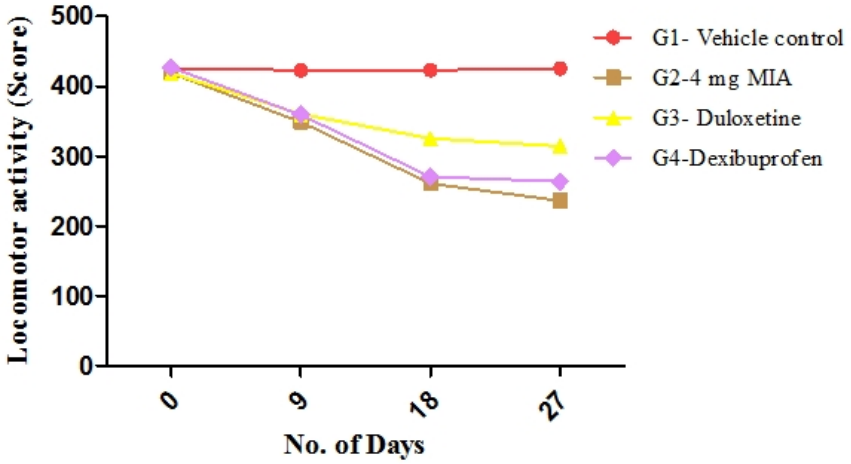

Fig. 8. Comparative effect of Duloxetine and Dexibuprofen on locomotor activity in MIA induced OA and depression in rats

Duloxetine has shown significant ($p<0.001$; Figs. 7 & 8.) antidepressant activity from day 9 to day 28, whereas dexibuprofen has shown comparatively lesser effect. Though NSAIDs has no reported antidepressant effect, the beneficial effect of dexibuprofen seen in this study could be due to its potent analgesic effect that might have enhanced the threshold for depression. There are some scientific publications on NSAIDs that support this claim. Endotoxins are potent stimulators of the synthesis and release of pro-inflammatory cytokines, and acute exposure induces several pronounced behavioral alterations, referred to as “sickness behaviors” [29,30].

In rodents, sickness behaviors include anhedonia, increased sleep, and decreased food intake, body weight, locomotor activity, social interaction, sexual behavior, and grooming. Further, the degree of overlap between flu-like syndrome and depression in humans is significant and a close linkage between these has been predicted to arise due to hypersecretion of pro-inflammatory cytokines [31-33]. In support of this, it has been reported that conventional antidepressants (i.e. selective serotonin reuptake inhibitors and tricyclics) may produce ameliorative effects by suppressing pro-inflammatory cytokine production [34-35]. Recent evidence suggests that NSAIDs also attenuate neuroimmune and neuroendocrine activation. Of particular interest, studies using rats have revealed that the NSAID zaltoprofen reduce sickness behavior and body weight loss induced by concanavalin-A (an activator of T-cells and cytokines) [36].

The significantly ($p<0.001$; Fig. 9.) increased weight gain noticed with the duloxetine, could be due to its effect on normalizing the serotonin level and analgesic effect as reported earlier [37-39]. The current study reveals that the antidepressants have the potential to be a useful adjuvant in the treatment of chronic OA and NSAIDs can potentiate the antidepressant effect. More studies have to be done to analyse other potential antidepressants alone and in combination with NSAIDs to address the complexity of symptoms associated with chronic OA.

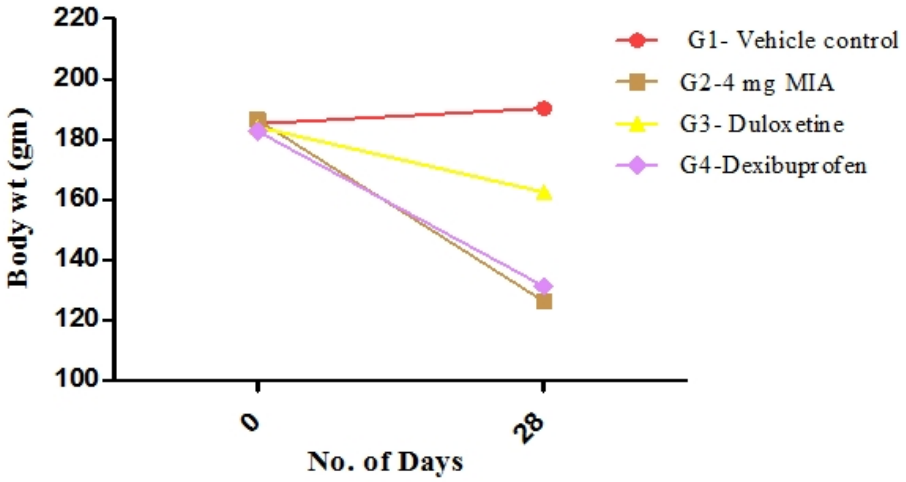

Fig. 9. Comparative effect of Duloxetine and Dexibuprofen on animal body weight in MIA induced OA and depression in rats

4. CONCLUSION

Chronic OA is a complex disease associated with neuropathic, nociceptive and depressive mechanisms. Currently approved therapies address either of these and not completely. The present study has shown that the NSAIDs have the potential in the initial phase of chronic OA pain and duloxetine in the later stage, where neuropathic and depressive component dominates.

CONSENT

Not aplicable

ETHICAL APPROVAL

Not applicable

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Sarzi-Puttini P, Cimmino MA, Scarpa R, Caporali R, Parazzini F, Zaninelli A. Osteoarthritis: an overview of the disease and its treatment strategies. Semin Arthritis Rheum. 2005;35:1–10.
2. Blay SL, Andreoli SB, Gastal FL. Chronic painful physical conditions, disturbed sleep and psychiatric morbidity: results from an elderly survey. Ann Clin Psychiatry. 2007;19:169–174.

3. Haack M, Lee E, Cohen DA, Mullington JM. Activation of the prostaglandin system in response to sleep loss in healthy humans: potential mediator of increased spontaneous pain. Pain. 2009;145:136–141.
4. Tiede W, Magerl W, Baumgärtner U, Durrer B, Ehlert U, Treede RD. Sleep restriction attenuates amplitudes and attentional modulation of pain-related evoked potentials, but augments pain ratings in healthy volunteers. Pain. 2010;148;36–42.
5. Korzeniewska-Rybicka I, Panik A. Analgesic Effect of Antidepressant Drugs. Pharmacol Biochem Behav. 1998;59:331-338.
6. Tura B, Tura SM.The analgesic effect of tricyclic antidepressants. Brain Res. 1990;518(1–2):19-22.
7. Peter C, Watson. Antidepressant drugs as adjuvant analgesics. J Pain Symp Manag. 1994;9(6):392-405.
8. Bannwarth B. Antidepressants in rheumatic disorders: do they act as analgesics or antidepressants? Joint Bone Spine. 2005:72(5):351-353.
9. Hawel RG, Singer F. Composition of the efficacy and tolerability of Dexibuprofen and Celecoxib in the treatment of osteoarthritis of the hip. In J Clin Pharmacol Ther. 2003;41:153-164.
10. Chappell AS, Ossanna MJ, Liu-Seifert H, Iyengar S, Jarevski S, Li LC, Bennett RM, Collins H. Duloxetine, a centrally acting analgesic, in the treatment of patients with osteoarthritis knee pain: A 13-week, randomized, placebo-controlled trial. Pain. 2009;146(3):253-260.
11. Grégoire S, Michaud V, Chapuy E, Eschalier A, Ardid D. Study of emotional and cognitive impairments in mononeuropathic rats: Effect of duloxetine and gabapentin. Pain. 2012;153(8):1657-63.
12. Robinson M, Perahia D, Pritchett Y, Raskin J. Duloxetine efficacy on pain symptoms: An independent analgesic effect. J Pain. 2006;7(4):S3.
13. Combe R, Bramwell S, Field MJ. The monosodium iodoacetate model of osteoarthritis: a model of chronic nociceptive pain in rats? Neurosci Lett. 2004;370(2-3):236-40
14. Kalbhen DA. Chemical model of osteoarthritis-a pharmacological evaluation. J Rheumatol. 1987;14:130-1.
15. Bove SE, Calcaterra SI, Brooker ML, Huber CM, Guzman RM, Juneau PL. Weight bearing as a measure of disease progression and efficacy of anti-inflammatory compounds in a model of monosodium iodoacetate-induced osteoarthritis. Osteoarthritis and Cartilage. 2003;11:821–830.
16. Fernihough J, Gentry C, Malcangio M, Fox A, et al. Pain related behavior in two models of osteoarthritis in the rat knee. Int Asso Study Pain. 2004;112;83-93.
17. Yu Y, Koo S, Kim CH, Lyu Y, Grady JJ, Chung JM. Two variables that can be used as pain indices in experimental animal models of arthritis. J Neurosci Method. 2002;115:107-103.
18. Karel-Martijn K , Mohammed EM , Janvan E et al. Pre-treatment with capsaicin in a rat osteoarthritis model reduces the symptoms of pain and bone damage induced by monosodium odoacetate. Eur J Pharmacol. 2010;1:108-13
19. Choi JI, Kim WM, Yoon MH, Lee HG, Antiallodynic Effect of Thalidomide and Morphine on Rat Spinal Nerve Ligation-induced Neuropathic Pain. Korean J Pain. 2010;23(3):172–8.
20. Dias JP, Ismael MA, Pilon M, et al. The kinin B1 receptor antagonist SSR240612 reverses tactile and cold allodynia in an experimental rat model of insulin resistance. Br J Pharmacol. 2007;152(2):280–7.
21. Niklas S, Jason J. Grading of monosodium iodoacetate-induced osteoarthritis reveals a concentration-dependent sensitization of nociceptors in the knee joint of the rat. Neurosci Lett. 2009;465:184–8.

22. Hochman JR, Gagliese L, Davis AM, Hawker GA. Neuropathic pain symptoms in a community knee OA cohort. Osteoarthritis and Cartilage. 2011;19(6):647-65.
23. Lin EH. Depression and Osteoarthritis. Tam J Med. 2008;121(11):S16-9.
24. Saxne T, Lindell M, Mansson B, Petersson IF, Heinegard D. Inflammation is a feature of the disease process in early knee joint osteoarthritis. Rheumatol. 2003;42:903–4.
25. Bajaj P, Graven-Nielsen T, Arendt-Nielsen L. Osteoarthritis and its association with muscle hyperalgesia: an experimental controlled study. Pain 2001;93:107–14.
26. Wessel J. The reliability and validity of pain threshold measurements in osteoarthritis of the knee. Scand J Rheumatol. 1995;24:238–42.
27. Schaible HG, Ebersberger A, Von Banchet GS. Ann N Y. Acad Sci. 2002;966(3):43–54.
28. Decosterd I, Woolf CJ. Spared nerve injury: an animal model of persistent peripheral neuropathic pain. Pain. 2000;87:149–58.
29. Konsman JP, Parnet P, Dantzer R. Cytokine-induced sick-ness behaviour: mechanisms and implications. Trends Neurosci. 2002;25:154–9.
30. Larson SJ, Dunn AJ. Behavioral effects of cytokines. Brain Behav Immunol. 2001;15:371–87.
31. Anisman H, Merali Z. Cytokines, stress, and depressive illness. Brain Behav Immunol. 2002;16:513–24.
32. Licinio J, Wong ML. The role of inflammatory mediators in the biology of major depression: central nervous system cytokines modulate the biological substrate of depressive symptoms, regulate stress-responsive systems, and contribute to neurotoxicity and neuroprotection. Mol Psychiatry. 1999;4:317–27.
33. Maes M. Major depression and activation of the inflammatory response system. Adv Exp Med Biol. 1999;461:25–46.
34. Castanon N, Leonard BE, Neveu PJ, Yirmiya R. Effects of antidepressants on cytokine production and actions. Brain Behav Immunol. 2002;16:569–74.
35. Lanquillon S, Krieg JC, Bening-Abu-Shach U, Vedder H. Cytokine production and treatment response in major depressive disorder. Neuropsychopharmacology. 2000;22:370–9.
36. Okamoto T. NSAID zaltoprofen improves the decrease in body weight in rodent sickness behavior models: proposed new applications of NSAIDs. Int J Mol Med. 2002;9:369–72.
37. Neupert W, Brugger R, Euchenhofer C, Brune K, Geisslinger G. Effects of Ibuprofen enantiomers and its coenzyme A thioester on human prostaglandin endoperoxide synthases. Br J Pharmacol. 1997;122:487-92.
38. Wurtmann JJ. Depression and weight gain: the serotonin connection. J Affect Dis. 1993;29:183-192.
39. Lake JK, Power C, Cole TJ. Back pain and obesity in the 1958 British birth cohort; cause or effect? J Clin Epidemiol. 2000;53(3):245-50.

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