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NORTRYPTYLINE ACT AS A POTENTIAL ADJUVANT TO DEXIBUPROFEN IN THE PREVENTION OF MIA INDUCED OSTEOARTHRITIS IN RAT MODEL OF CHRONIC PAIN AND PAIN INDUCED DEPRESSION.

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ABSTRACT

Osteoarthritis (OA) commonly known as wear and tear arthritis is more prevalent due to increasing aged population and obese people worldwide. OA is also associated with depression. Effective therapy regimen for the OA addressing both pain and depression is limited. Therefore, research on OA is need of the hour. Non-Steroidal anti-inflammatory drugs (NSAIDs) are the conventional OA treatment and TCAs has been extensively studied as adjuvant to enhance analgesic effect. As TCAs has shown analgesic effect and basically they are antidepressants, the synergistic effect of Nortryptyline (TCAs) with dexibuprofen (NSAID) and individual drugs has been evaluated in mono-sodium Iodo acetate (MIA) induced OA in rats for its analgesic and antidepressant effects. The results have shown that the combination of dexibuprofen and nortryptyline has synergistic analgesic effect and in addition relieved the chronic pain induced depression.

KEY WORDS: Osteoarthritis, depression, MIA, adjuvants, Nortryptyline and Dexibuprofen

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INTRODUCTION

Osteoarthritis (OA) is the major chronic irreversible degenerative joint disease which is more prevalent due to increasing aged population and obese people worldwide. Knee joint is the most commonly affected and the greater risk of disability among all joints, since it is the major weight bearing joint[1]. It has also been reported that people with OA are more prone to psychological depression. The causes of these two disease states have common neuromodulators and neurotransmitters acting within the hippocampus. Hippocampus is the well accepted region of the brain which involved in depressive illness and pain processing [2]. The effective therapy regiment for the osteoarthritis is limited. The conventional treatment like non-steroidal anti-inflammatory drugs, opioids and steroids has shown only limited alleviation in chronic OA. As such this conventional treatment does not offer any benefits for chronic associated depression, which is a major concern. Because chronic pain and chronic pain induced depression involves multiple mechanisms, the research on combination therapy is rationale and need of the hour. The purpose of this present study is to investigate the new adjuvant therapy Nortryptyline, a tricyclic antidepressants (TCA) along with the Dexibuprofen, a Non-steroidal anti-inflammatory drug (NSAID), which is the conventional OA treatment with fewer side effects. Dexibuprofen, which is the active enantiomer (S-Ibuprofen) of Ibuprofen, is about 160 times more potent than R-Ibuprofen as observed during *invitro* inhibition of prostaglandin synthesis [3]. It has also shown that the Gastro-intestinal toxicity of Dexibuprofen is lower than that of Ibuprofen [4].It also has a better tolerability when compared with other NSAIDs [5, 6]. Most animal studies involving acute nociceptive tests or persistent pain models (neuropathic or nociceptive) have concluded that anti-depressants have an antinociceptive effect or an antihyperalgesic effect [7]. TCA has been extensively studied and were originally developed as a tuberculosis remedy and as an antihistamine, respectively. These drugs were later found to have an antidepressant effect in the 1950s and shown to increase synaptic levels of noradrenaline (NA) and 5-hydroxytryptamine (5-HT). Further, it has been shown that antidepressants modulate not only the monoamine neurotransmitter system but also the inflammatory system [8]. The first evidence indicating that antidepressants have antiinflammatory effects appeared four decades ago. Martelli et al. (1967) showed that administration of TCAs inhibited chemically induced edema in the standard rat paw assay[9]. Nortryptyline is the major metabolite of amitriptyline the most widely used drug for which an effect on pain, fatigue, sleep and general condition has been demonstrated [7]. The analgesic effect of antidepressants was studied but there was no information available about the consequences on pain associated depression. Therefore this study was designed to assess the synergism of NSAIDs and TCAs in the management of pain and its associated depression in OA. There is no such combination targeting both pain and its associated depression in OA till date.

MATERIALS AND METHODS

Experimental animals and induction of arthritis

Animals

Healthy male wistar rats weighing about 150 – 200 g were obtained from institute animal Center. The protocol was approved by the institute's animal ethical committee (IAEC no. 126/bc/09/CPCSEA). Animals were kept in uniform husbandry conditions and given pelleted diet (lipton's india Ltd.) and water ad libitum.

Experimental design

Thirty male wistar rats , were used in this study. The rats were kept in a room with a reversed 12-h light/dark cycle (08:00–20:00 dark cycle), and free access to food and water. Rats were housed for a minimum of 7 days in this

environment. The animals were divided into 5 groups of 6 animals each as follows

Group 1: Vehicle control (50µl of 0.9% normal saline, I.A. injection)

Group 2: Osteoarthritis control (50µl of MIA, I.A. injection)

Group 3: MIA (50µl) + Nortriptyline (0.5/ mg/kg, t.i.d)

Group 4: MIA (50µl) + Dexibuprofen (30 mg/kg, t.i.d)

Group 5: MIA (50µl) + Dexibuprofen (30 mg/kg, t.i.d) + Nortriptyline (0.5/ mg/kg, t.i.d)

The drug treatment is started on day 1 and continued upto day 28.

Induction of osteoarthritis

MIA (80mg/ml) was dissolved in 0.9% sterile normal saline. Prior to inducing OA, rats were anaesthetised with ketamine (50mg/kg,i.p) . After appropriate anaesthesia each rat was positioned on its back and the right leg was flexed 90 degrees at the knee. The day of OA induction was considered as day 0. Test group animals are treated with MIA 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. The patellar ligament was palpated below the patella and the injection was made into this region. Care was taken while injection, because the needle is not to advance in too far into the cruciate ligaments. 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[7-9]. The animals will be monitored for osteoarthritis 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.

METHODS

Osteoarthritis activity

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 (mm)[10-11]

Evaluation of Mechanical Hyperalgesia:

The vocalisation threshold was determined by compression of the knee joint. The vocalisation threshold of knee compression was measured with the help of forceps which having two the length of 20 cm long and 4mm X 4mm contact area. It was fitted with pressure gauges which produce an output voltage that is potential to the applied force. The pressure was applied continuously over the knee joint until an audible squeak was elicited. The output voltage was calibrated as grams of force using a known weight suspended by a string which was read at the time of vocalisation. Since repeated application of pressure in short term intervals may sensitize the knee. Vocalisation threshold was measured once at each time point[11]

Measurement of Punctate allodynia

Punctate allodynia was assessed by measuring withdrawal thresholds to calibrated von Frey hairs. A maximal cut off of 15 g was used because normal rats do not respond to this level of tactile stimulation, and thicker filaments (>15 g) lift the hind paw from the wire mesh, thus not accurately testing paw withdrawal thresholds. 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 min before pain behaviour assessment. Static allodynia was evaluated by application of von Frey hairs in ascending order of force for up to 6 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. Three readings were taken for each rat at each time point (at least 1min elapsed between each test), and the average was determined and used for subsequent analyses[10, 12-14].

Assessment of Grip strength

Grip strength and muscle co-ordination was assessed by using Rota rod instrument. The duration of animal grasping the revolving rod (10 cm diameter; 16 rounds per min, 20 rpm)

was recorded either by manually or automatically depending upon the instrument. The cut off time for this test is 180 sec. Before going to perform this experiment animals are accustomed by this test. The trial was conducted 5 times for each rat and then the mean riding time was found out. During the time of riding the animal doesn't fall within 180 sec the animal was released from the revolving rod[12-15].

Weight bearing

Changes in hind paw weight distribution between the right (osteoarthritic) and left (contralateral control) limbs were utilized as an index of joint discomfort in the osteoarthritis knee. 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-s period. Each data point is the mean of three, 5-s 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.

Struggle threshold angle of knee extension

Reduced range of motion and mechanical sensitivity of the arthritic knee have been assessed by measuring the struggle threshold of the knee extension angle, 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 thigh was fixed by holding it with the thumb and the second finger of one hand. Using the fingers of the other hand, the leg was extended to determine the knee extension angle at which the rat showed struggling behaviour. To do this, the distance that the heel of the foot travels during the extension was measured . The extension angle was then calculated by trigonometric function using the length of the tibia and the foot travel distance during extension. Since this procedure can be repeated without sensitizing the knee, measurements were repeated three times at 3-min intervals and the average of the three was taken as the final value[11].

DEPRESSENT ACTIVITY

Depressant and psychological activity is assessed by following Forced swim test,Actophotometer on every alternate day.

Forced swim test

The Forced swim test (FST) was carried out according to Porsolt etal. This test is most widely used and recognized pharmacological model for assessing antidepressant activity. The development of immobility when the rats are placed in an inescapable cylinder filled with water reflects the cessation of persistent escape-directed behavior. The apparatus consisted of a clear Plexiglas cylinder (30 cm high x22cm diameter) filled with water up to 15 cm, maintained the temperature at 23-25ºC. In the pre-test session, every animal was placed individually into the cylinder for 15 min for training; after 24 hrs the immobility test was conducted for 5 min. Scores of each animal were recorded. During the test session the following behavioural responses were recorded: climbing behavior, which is defined as upward-directed movements of the forepaws along the side of the swim chamber; swimming behavior, defined as movement throughout the swim chamber, which include crossing into another quadrant; and immobility time, that was considered. The rat was judged immobile if it 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[12-14].

Spontaneous Locomotor Activity

To evaluate spontaneous locomotor activity, each animal was individually placed in an actophotometer. The locomotor activity can be an index of wakefulness of mental activity. Most of the central nervous system acting drugs influence the locomotor activity in man and

animals. The CNS depressants reduce the motor activity while the stimulants increase the activity. The photocells of the actophotometer were checked before use and the animals were individually placed in a square arena (30 x 30cm). After an initial accustomed period (2 min), the locomotor scores were recorded digitally for the next 10 min[13-14].

Measurement of Body weight

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

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). A *P*-value of less than 0.05 was considered to be significant.

RESULTS

Figure-A represents the measurement of knee joint diameter. On day 1, the rise in inflammation is almost similar in all MIA treated groups ($p<0.01$). On day 3, the inflammation has increased significantly in Group-II, and reduced inflammation was noticed from Group-III to V ($p<0.001$). The inflammation has reached near to normal on 7 th day in all treatment groups compared to positive control with more reduction in Group-V. Mechanical hyperalgesia was shown in Fig B. The MIA induced group had shown significant ($p<0.001$) increase in Vocalization threshold of knee compression force, the mechanical hyperalgesia as compared to vehicle control group. In contrast, animals treated with Dexibuprofen + Nortryptyline had shown significant ($p<0.001$) inhibition in mechanical hyperalgesia compared to MIA induced animals. The paw withdrawal threshold has significantly decreased in group II ($p<0.001$) compared to Group I (Fig C). The threshold has increased in treatment groups and it is more prominent in Group V ($p<0.001$). Figure D represents the result of mechanical grip strength. Group V showed significantly ($P<0.05$) increased the mechanical gripstrength as compared to Group II-IV. The hind paw weight distribution experiment (Fig E) has shown no difference in weight distribution in hind paws of Group-I, whereas the difference is increased in Group-II ($p<0.001$), and among the treatment groups, Group-V has significantly increased the threshold ($p<0.001$). The threshold angle of knee extension has been depicted in Fig-F. The combination therapy (Group-V) was significantly ($p<0.001$) effective in improving the threshold compared to other treatment groups. Figure-G & H shows the results of locomotor activity. Group-V has shown good antidepressant activity compared to other treated groups. There was significant ($p<0.001$) reduction in body weight (Fig-I) in disease induced animals (Group-II). Drug treated animals (Group-III to V) improved the body weights, where the increase is significantly ($p<0.001$) higher in Group-V.

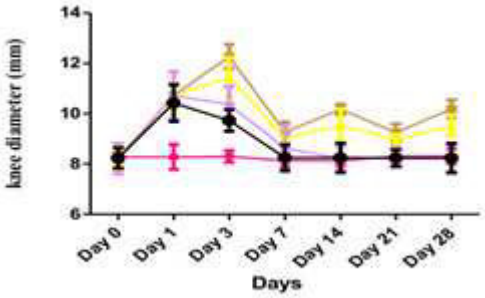
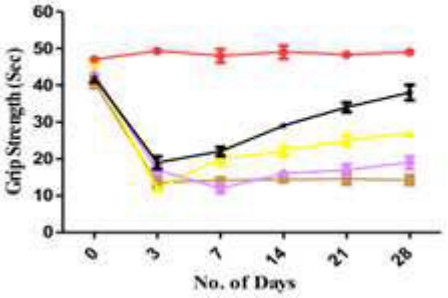


Figure : A



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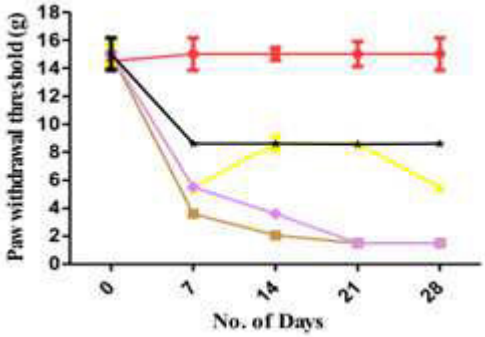

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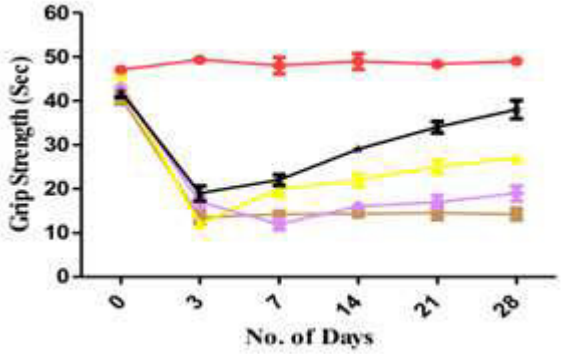
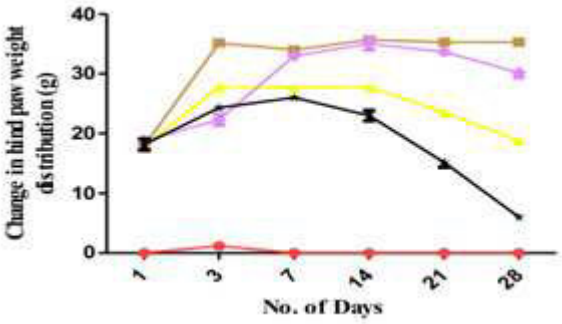


Figure : D



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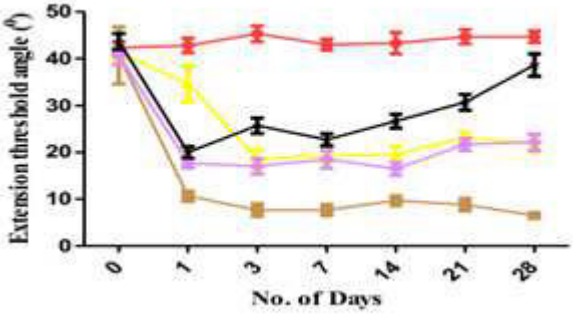
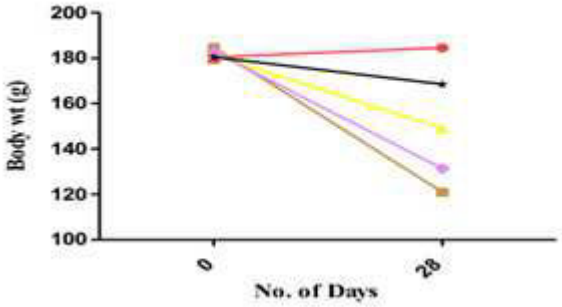


Figure : F



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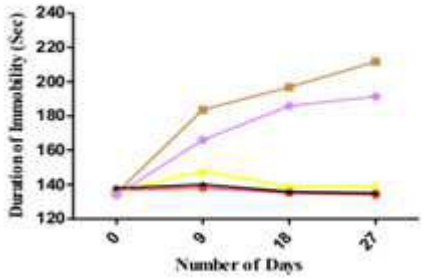

Figure : H

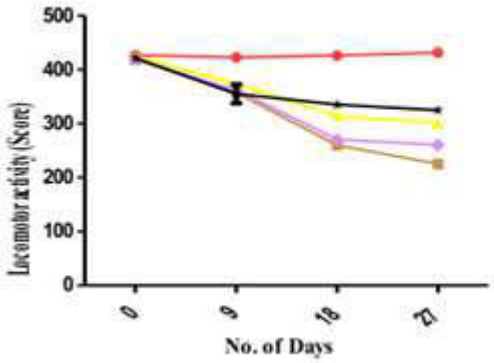

Figure : I

Figure A to I: Comparative effect of Nortryptyline, and Dexibuprofen alone and in combination against MIA-induced osteoarthritic chronic pain and pain induced depression in rats. Values are in MeanÂ±SEM. Figure A to I represents Knee diameter (A), Mechanical hyperalgesia (B), Dynamic allodynia (C), Grip strength (D), Weight bearing threshold (E), Knee extension threshold (F), Forced swim test (G), Locomotor activity (H) and Body weight (I), respectively.
In individual graph, Group I to V represents, Circle (Group I), Square (Group II) , Triangle (Group III), Collate (Group IV) and Star (Group V), respectively.

DISCUSSION

Intra-articular injection of MIA into rat knee joints induce histological changes and pain related behaviours characteristic of human OA[8,9,15]. Most of the MIA-induced studies have focused on histological changes of the joint and related pain behaviour but not much is known about the pain induced depression. There is an intimate relationship of pain and depression, and rheumatologist has always been aware of the importance of mood disturbance in rheumatic patients. In various pain clinics, depression has been present in approximately 40 to 60% of patients. [19-20] The present study has evaluated the potential of Tricyclic antidepressants (Nortryptiline) and NSAIDs (Dexibuprofen) combination for the treatment of OA induced chronic pain and pain induced depression in a MIA induced OA model. Analysis of 39 studies in various chronic non-malignant painful conditions[16] found that antidepressants had an analgesic effect, confirmed by Lynch in a meta-analysis of 59 studies.[17] In global assessment of effects of antidepressants in chronic pain states, most of the authors have concluded that the tricyclic group of antidepressants is analgesic.[18] Therefore, nortryptyline which is basically antidepressant also has analgesic properties. Combining this drug with NSAID, would be

synergistic for analgesic properties and exert antidepressant effects also.

With respect to, highly COX-2 selective drugs like Rofecoxib, Etoricoxib, Valdecoxib exhibit notable cardiotoxicity whereas highly COX-1 selective drugs like Ketorolac, Flurbiprofen exhibit profound GI toxicity. But drugs like Naproxen, Ibuprofen, Diclofenac, Piroxicam, which have COX-2/COX-1 ratio closer to 1, seem to have less GI and CV risk. COX-2/COX-1 ratios emphasize the importance of a balanced inhibition of both enzymes by NSAIDs to achieve an acceptable safety profile[21].In relation to that, in general, ibuprofen has the lowest risk among older NSAIDs[22-23]. As per the latest evolving information, compared to Ibuprofen, its active enantiomer S-Ibuprofen (Dexibuprofen) has low GI toxicity. More interestingly, COX-2/COX-1 ratio of Ibuprofen and Dexibuprofen has been evaluated by the reliable human whole blood assay method, and surprisingly COX-2/COX-1 ratio for Dexibuprofen is closer to one (0.76) and that for Ibuprofen is 7.2[24]. Hence among available NSAIDs , Dexibuprofen seems to be the safe NSAID. As previously reported (Fernihough J et.al, 2004)[10] brief period of inflammation was noted after MIA injection, characterised by an increase in knee diameter to 12.27 mm ($p<0.001$for difference from vehicle controls) at day three post injection, which was reduced to normal levels by day seven. Combination of Nortryptyline + Dexibuprofen decreased the knee inflammation [Fig-A] significantly ($p<0.001$) from day 3 to day 7, and does not show any increase in inflammation till day 28. The results are similar for Dexibuprofen but not for Nortryptyline. Also, slight fluctuation is seen every 7 days after day 7 in Group III but not in Dexibuprofen treatments. This shows that NSAID has a major role in reducing OA induced inflammation than TCAs. Dexibuprofen reduced hyperalgesia at day3[Fig-B] following MIA injection, when there is measurable knee joint swelling, suggesting an early component of inflammatory pain in this chemically induced model. This is similar to the previous reports on NSAIDs.[10]. Nortryptyline effect increased after day seven, whereas Dexibuprofen has no effect after day 7, and the combination Nortryptyline + Dexibuprofen, significantly ($p<0.001$) reduced hyperalgesia at all time points. The lack of effect of Dexibuprofen at days 14 and 28 argues that non-inflammatory mechanisms are responsible for the pain behaviours at later time points.

Punctate allodynia was detected in all MIA-treated rats from day 7 until day 28, with all animal demonstrating PWT to the previously innocuous 4.0g or below(Fig.C)as compared to the normal PWT of 15.0g. Neither the contra lateral paw of MIA-treated nor the control group animals showed changes from base line at any time point. Punctate allodynia is signalled by Aδ primary sensory neurones. In humans, the origin of OA associated joint pain is thought to be due to the stimulation of C-fibres and Aδ nerve endings in the Synovium and surrounding joint structures, such as ligaments and muscles [7]. The combination of Nortryptyline + Dexibuprofen significantly ($p<0.001$) increased the threshold at all time points demonstrating the combination effect on OA associated joint pain through Aδ primary sensory neurons. Grip strength reduced from day 3with all MIA treated rats and it significantly ($p<0.001$) increased in Nortryptyline + Dexibuprofen group to 38 sec compared to 14 sec in Group-II (Fig.D) . A similar response is noticed with weight bearing, where a significantly ($p<0.001$) increased weight bearing capacity (6 g) was noticed for the combination (Group V), compared to 35g in Group-II (Fig.E). The same is the response for combination in enhancing knee extension threshold (Fig.F) . The change in hind paw weight distribution over time closely follows that of punctate allodynia. Also, the lack of effect for dexibuprofen in grip strength, weight bearing and knee extension at later time points suggest a potential neuropathic pain component of MIA-induced pain at these time-points.

A significant ($p<0.001$) antidepressant activity has been noticed for Nortryptyline + Dexibuprofen in forced swim test (Fig.G) and locomotor activity (Fig.H). This is the kind of response every chronic pain patients require to completely address the symptoms in many perspective. In correlation with the beneficial analgesic and antidepressant effect observed

for the Nortryptyline + Dexibuprofen, there is significant ($p<0.001$) impact on weight gain (168.42 g) (Fig.I) compared to MIA-induced group (120.92 g). As per a survey reported by Wurtman JJ,1993 , 60% of the obese also reported increased depression and fatigue[24] .Brain serotonin appears to be involved in these disturbances of mood and appetite[25].In addition, elevated risk of pain has also been observed to be related to obesity, especially among women[26]. The controlled weight gain noticed with the Nortryptyline + Dexibuprofen and nortryptyline, could be due to its effect on normalizing the serotonin level and analgesic effect. Antidepressants have become, common drugs for the treatment of chronic, mainly neuropathic, pain, even though their efficacy is limited. Research in the field of antidepressants has evolved substantially in recent years but there are still many issues to be elucidated in both preclinical and clinical research [27].

CONCLUSION

Osteoarthritis leads to chronic pain and depression. Combination of antidepressant drug as potential adjuvant to NSAIDs has the potential to address both. The present study has shown that the combination of dexibuprofen and nortryptyline has the significant potential to treat OA induced chronic pain and depression.

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