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Effect of alcoholic extract of *Entada pursaetha* DC on monosodium iodoacetate-induced osteoarthritis pain in rats

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***Background & objectives*: Osteoarthritis (OA) is a degenerative disease characterized by joint pain and progressive loss of articular cartilage. *Entada pursaetha* has been traditionally used in the treatment of inflammatory disease, liver ailment, *etc*. In this study we investigated suppressive effect of ethanolic extract of *E. pursaetha* (EPE) on monosodium iodoacetate (MIA)-induced osteoarthritis pain and disease progression by histopathological changes in joints in a rat model.**

***Methods*: OA was induced in right knee of rat by intra-articular injection of 3 mg of MIA and characterized by pathological progression of disease and pain of affected joint. Spontaneous movements, mechanical, thermal and cold sensitivity were monitored at days 0 (before drug and MIA injection), 7, 14 and 21 of MIA administration. EPE (30, 100 and 300 mg/kg), vehicle or etoricoxib (10 mg/ kg; reference drug) were administered daily for 21 days by oral route.**

***Results*: EPE at various doses significantly reduced mechanical, heat, cold hyperalgesia and increased the horizontal and vertical movements in intra-articular MIA injected rats. EPE prevented the damage to cartilage structure and reduced the cellular abnormalities. Articular cartilage of rats treated with EPE at 300 mg/kg group was almost normal with well-developed smooth surface and chondrocytes were distributed individually or arranged in column.**

***Interpretation & conclusions*: The present findings showed that the EPE was not only able to mitigate pain and hyperalgesia but also inhibited MIA-induced cartilage degeneration *in vivo*. EPE may have the potential to become therapeutic modality in the treatment of osteoarthritis. However, further studies need to be done to confirm these findings in other models and clinical trials.**

Key words *Entada pursaetha* - hyperalgesia - monosodium iodoacetate - osteoarthritis - pain

Osteoarthritis (OA) is a chronic joint disease characterized by progressive cartilage degeneration, synovial inflammation, subchondral bone sclerosis, and osteophyte formation[1]. A complex network of cytokines and proteolytic enzymes leads to degradation of the extracellular matrix (ECM) proteins of cartilage such as type II collagen (CII), proteoglycans and hyaluronic acid[2]. Although studies have confirmed

the efficacy of non-steroidal anti-inflammatory drugs (NSAIDs) and anti-cycloxygenase-2 (COX-2) as symptomatic treatments for OA, these drugs have not proven to positively affect the natural course of OA in humans[3]. In this context, diacerein has been shown as an attractive candidate[4]. Monosodium iodoacetate (MIA)-induced OA model used in this study mimics severe and acute OA pain which is similar with human OA[5]. The MIA-induced OA model is highly reproducible with predictable pain behaviour responses.

Entada pursaetha, a large gigantic woody climbing shrub (liana) among legumes is widely distributed in tropical Africa, India, China, Philippines, Guam and Northern Australia[6]. In India, it is found in damp forest of Bengal, Bihar and Odisha, in forest region of Eastern and Western Ghat and hilly forest tract of northern district of Bengal and Deccan[7]. Mainly seeds, leaves and stem or stem bark of *E. pursaetha* are used for different medicinal purposes.

Antibacterial, antiviral, analgesic, anti-inflammatory and hypoglycaemic activities of plant extracts have been studied earlier[8]. Free radical scavenging activity of leaf extract[9] and anti-inflammatory and hepatoprotective activity of seed extract of *E. pursaetha* have also been reported[10,11]. But no study has been done on its anti-arthritic or anti-osteoarthritis activity. In the present study, we investigated whether ethanolic extract of *E. pursaetha* (EPE) stem would suppress OA pain and its progression by examining behavioural pain parameters and histopathological changes elicited in MIA-induced experimental OA rat model.

Material & Methods

This study was conducted in the division of Pharmacology and Toxicology, Indian Veterinary Research Institute (IVRI), Bareilly, Uttar Pradesh, India. Male Wistar rats (Livestock Resource Section, IVRI weighing 140-175 g at the time of surgery for the induction of model were used. They were housed at a maximum of four per cage on a 12-hour day/night cycle at a temperature of 22±1°C. Water and food were provided *ad libitum*. All animal experimental procedures were duly approved by the Animals Ethics Committee of IVRI.

Preparation of alcoholic extract of E. pursaetha: The mature stem of *E. pursaetha* was brought from the jungles of Bhawanipatna, district - Kalahandi, Odisha, India, and authenticated by Dr B.N. Pandey, Department of Botany, Bareilly College, Bareilly (India). A voucher specimen (023/09) was deposited in the Indigenous Drug laboratory of division of Pharmacology & Toxicology. The powdered stem was refluxed twice with 85 per cent ethanol at 95°C for 8 h. Ethanol was removed under vacuum and solid extract of *E. pursaetha* stem was obtained (henceforth referred as EPE). The yield of the extract was 8.4 per cent with reference to dry starting material. EPE was used by suspending in maximum of 0.2 per cent polysorbate 80. Three different doses of EPE 30, 100 and 300 mg/kg body weight were used, based on the earlier work carried out in our laboratory (unpublished data).

Estimation of phytoconstituents of EPE - EPE was made to the concentration of 100 mg/ml in ethanol and this was used as stock solution for the quantitative phytochemical estimation.

Estimation of total phenolic content - Total phenols were determined as described earlier[9]. In brief, 0.5 ml of plant extract was mixed with 5 ml Folin Ciocalteu reagent (SRL Pvt. Ltd, India) (1:10 diluted with distilled water) and aqueous 4 ml, 1 M sodium carbonate. The mixtures were allowed to stand for 15 min and the total phenols were determined by colorimetry at 765 nm. The standard curve was prepared using 100, 50, 25 and 12.5 µg/ml solution of gallic acid in methanol: water (50:50, v/v). Total phenol values were expressed in terms of gallic acid equivalent mg/g of extract.

Estimation of total tannin content - Stock ethanolic extract (0.1ml) was mixed with 0.5 ml of Folin-Denis reagent (Sigma Aldrich, USA) followed by 1 ml of Na_2CO_3 (0.5% w/v) solution and made up to 10 ml with distilled water. The absorbance was measured at 755 nm within 30 min of the reaction against the reagent blank. Standard curve was prepared using 500, 250, 125 and 62.5 µg/ml tannic acid solution. Total tannins in extracts were expressed as equivalent to tannic acid (mg TE/g extract)[10].

Estimation of total flavonoid content - Total flavonoids were determined by aluminum chloride colorimetric method[11]. In brief, 0.5 ml of plant extract in methanol was separately mixed with 1.5 ml of methanol, 0.1 ml of 10 per cent aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. After keeping at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm with a double beam UV/Visible spectrophotometer. The calibration curve was prepared by preparing quercetin solutions at concentrations

100, 50, 25 and 12.5 µg/ml in methanol. Total flavonoids in extracts were expressed as equivalent to quercetin (mg QE/g extract).

Estimation of total saponin content - Total saponins were determined by vanillin and sulphuric acid method[12] in which 0.5 ml of plant extract was mixed with 0.5 ml of 8 per cent vanillin in ethanol and then 5 ml of 72 per cent v/v sulphuric acid in deionized water was added and mixed well in ice-water bath. The mixture was kept in hot water bath at 60°C for 10 min and cooled in ice cold water bath. Absorbance was taken at 460 nm. The standard curve for saponins was prepared by using saponins for quillaja bark (sapogenin) from Sigma at concentrations 1000, 500, 250 and 125 µg/ml in distilled water.

Induction of osteoarthritis: On day 0, rats were anaesthetized with ketamine hydrochloride (50 mg/kg body weight) and xylazine (10 mg/kg body weight) and the right knee was shaved and disinfected with 70 per cent ethanol followed by povidone-iodide. A single injection of 50 µl sterile normal saline containing 3 mg monosodium iodoacetate was injected into right knee joint through infrapatellar ligament using a 300 µl syringe fitted with a 29 G needle, as described earlier[13]. A pilot study was undertaken to induce osteoarthritis with MIA. On day 21 post MIA administration, the knee joint was examined histopathologically and cartilage was stained with special stain safranine-O for accessing whether osteoarthritis was produced or not[14]. After establishing MIA-induced osteoarthritis in pilot study, six experimental groups I, II, III, IV, V and VI, each consisting of six rats were taken. Animals of groups I and II were naive control and vehicle control, respectively. On day 0, rats of all groups except naïve control were administered with a single dose of MIA in their right knee joint cavity while naïve control was injected with 50 µl of normal saline only. From day 0 to 21, rats in groups III, IV, and V were administered EPE daily at 30, 100, and 300 mg/kg doses, respectively through oral gavage before MIA injection. Rats of groups I and II were administered normal saline and vehicle, respectively. Rats of group VI were administered with etoricoxib at10 mg/kg from day 0 to 21, daily orally. Pain assessment was done on days 0 (before administration of MIA), 7, 14, and 21 of experiment by measuring different parameters such as mechanical hyperalgesia, thermal hyperalgesia and cold hypersensitivity, spontaneous motor activity. Knee diameter was taken on day 0 and 21. On day 21, after 3 h of EPE administration rats of all groups were sacrificed by cervical dislocation and right knee was collected for histopathological examination.

Behavioural assessment of nociception: Pain assessment was done on days 0 (before EPE/etoricoxib/vehicle or normal saline administration), 7, 14, and 21. Rats were habituated to the testing situation for at least one week before the start of experiment and 5 to 10 min before each testing until the exploration activity ceases.

Secondary mechanical hyperalgesia: Secondary mechanical hyperalgesia was assessed by the Randall-Selitto test[15] using an analgesimeter/Randall Selitto paw pressure (Ugo Basile, Varese, Italy) device. The rats were handled carefully and partly restrained. The hind paw was placed on platform under a cone-shaped pusher and then linearly increasing mechanical force was applied. The paw withdrawal threshold (PWT) was defined as the pressure in g at which the rat pulled out its paw. The cut-off pressure was set to 250 g.

Heat hyperalgesia: Sensitivity to heat was determined by measuring paw flick latency to infrared heat source (Tail Flick unit, Ugo Basil, Varese, Italy). Planter side of paw was placed above the heat source and paw withdrawal latency was noted in seconds. A maximum cut-off time of 20 sec was used to minimize paw damage. Measurements were taken 2-3 times with at least 5 min gaps between the tests.

Cold hypersensitivity: Paw withdrawal latency was recorded in sec by submerging ipsilateral and contralateral paw in cold water (4±1°C). Measurements were taken 2-3 times with at least 5 min gaps between the tests.

Spontaneous motor activity: Horizontal and vertical spontaneous motor activity of rats of each group was accessed by automatic activity cage (Ugo Basil, Varese, Italy) before recording pain parameters. An infrared beam cage, which consists of an animal cage of clear perspex, complete with two sets of emitter/sensor arrays for horizontal and vertical activity, respectively. The numbers of interruptions in beam path animal make while moving or standing on hind limbs are counted during five minutes.

Histopathological analysis: The right knee joints were dissected, fixed, and decalcified in 10 per cent formic acid for 72 h. The tissue sections (10 µm) were subjected to hematoxylin and eosin (H&E) staining for routine histological examination.

Statistical analysis: Statistical analysis of data was done by GraphPad InStat software version 4.00 (San

Diego, California, USA) using one way ANOVA with Tukey multiple comparison. All groups were compared with each other for significance.

Results

The concentration of saponin was highest (57.3 mg/g extract) followed closely by total phenols (47.6 mg/g extract). Total concentration of tannins and flavonoids was almost similar (13.72 and 13.1 mg/g extract, respectively).

Mechanical hyperalgesia: Paw withdrawal threshold (PWT) values of right paw of different groups on days 0, 7, 14 and 21 are summarized in Table I. Significant mechanical hyperalgesia was observed in ipsilateral hind paw in MIA-injected animals at all observed time points (days 7, 14 and 21) as compared to naïve group of rats. Treatment of MIA-injected rats with EPE 30, 100 and 300 mg/kg and etoricoxib 10 mg/kg induced a significant increase in PWT compared to MIA-injected vehicle - treated animals on days 7, 14 and 21.

Heat hyperalgesia: Paw withdrawal latency (PWL) in seconds to noxious heat of different groups on days 0, 7, 14 and 21 time points is summarized in Table I. On day 0, right paw withdrawal latency to noxious heat of different groups did not differ significantly. On days 7, 14 and 21, significant ($P<0.05$) heat hyperalgesia was observed in vehicle-treated control following MIA injection in right joint as compared to naïve control.

Table I. Effect of EPE (ethanol extract of *E. pursaetha*) on mechanical hyperalgesia, heat hyperalgesia, cold hyperalgesia in monosodium iodoacetate-induced osteoarthritis in rats

Group	Treatment (Dose in mg/kg)	Day after treatment			
		0	7	14	21
Mechanical hyperalgesia (paw withdrawal time in gm, mean ± SD					
I	Naïve control	61.67 ± 11.25[a]	62.50 ± 9.35[a]	74.17 ± 7.36[a]	80.00 ± 10.49[a]
I	Vehicle control	60.00 ± 9.38[a]	35.83 ± 3.76[b]	35.00 ± 4.47[b]	49.17 ± 5.84[b]
III	EPE (30)	69.17 ± 8.61[a]	74.17 ± 12.81[a]	95.00 ± 10.49[ac]	111.7 ± 10.32[c]
IV	EPE (100)	64.17 ± 7.36[a]	70.00 ± 13.04[a]	111.7 ± 20.66[c]	103.3 ± 10.80[c]
V	EPE (300)	60.83 ± 7.36[a]	81.67 ± 21.60[a]	109.2 ± 20.35[c]	117.5 ± 19.43[c]
VI	Etoricoxib (10)	66.67 ± 10.32[a]	81.67 ± 17.51[a]	79.17 ± 16.56[a]	75.83 ± 10.68[a]
Heat hyperalgesia (paw withdrawal latency in sec, mean ± SD)					
I	Naïve control	5.400 ± 0.72[a]	6.100 ± 0.45[a]	5.083 ± 0.43[a]	5.033 ± 0.28[a]
II	Vehicle control	5.333 ± 0.72[a]	4.017 ± 0.32[b]	3.750 ± 1.03[b]	3.733 ± 0.28[b]
III	EPE (30)	5.200 ± 0.82[a]	4.300 ± 0.77[b]	4.300 ± 0.77[b]	5.833 ± 0.39[a]
IV	EPE (100)	5.100 ± 0.81[a]	4.533 ± 1.60[b]	3.933 ± 0.95[b]	5.650 ± 0.58[a]
V	EPE (300)	4.533 ± 1.20[a]	6.650 ± 0.97[a]	5.217 ± 0.75[a]	6.867 ± 0.60[cd]
VI	Etoricoxib (10)	4.283 ± 0.80[a]	6.017 ± 0.40[a]	6.250 ± 0.42[a]	5.933 ± 0.99[ad]
Cold hyperalgesia (paw withdrawal latency in sec, mean ± SD)					
I	Naïve control	7.250 ± 0.92[a]	8.467 ± 1.97[a]	7.867 ± 1.60[a]	6.617 ± 0.49[a]
II	Vehicle control	8.567 ± 1.90[a]	3.833 ± 0.48[b]	3.833 ± 0.48[a]	3.900 ± 0.52[b]
III	EPE (30)	6.517 ± 1.95[a]	11.38 ± 1.04[ac]	12.23 ± 1.91[b]	8.917 ± 2.05[a]
IV	EPE (100)	7.367 ± 2.64[a]	11.28 ± 1.43[ac]	12.55 ± 1.29[b]	10.03 ± 2.09[c]
V	EPE (300)	9.100 ± 3.70[a]	10.92 ± 2.57[a]	12.02 ± 1.11[b]	10.07 ± 1.33[c]
VI	Etoricoxib (10)	8.033 ± 1.59[a]	12.87 ± 1.62[c]	8.283 ± 1.63[a]	7.517 ± 0.93[a]

(n = 6). All groups were compared with each other for significance; Values bearing no superscript in common differ significantly; $P<0.05$.

Table II. Effect of EPE (ethanol extract of *E. pursaetha*) on horizontal spontaneous movement and vertical spontaneous movement in monosodium iodoacetate-induced osteoarthritis in rats

Group	Treatment (Dose in mg/kg)	Day after treatment			
		0	7	14	21
Horizontal spontaneous movements (Number of interruptions/5 min; (mean ± SD)					
I	Naïve control	1140 ± 123.45[a]	1242 ± 144.91[a]	1310 ± 153.71[a]	1211 ± 85.48[a]
II	Vehicle control	1046 ± 126.32[a]	657.7 ± 129.21[b]	689.5 ± 170.05[b]	560.5 ± 164.81[b]
III	EPE (30)	1325 ± 209.35[a]	1075 ± 164.76[a]	1182 ± 91.87[a]	1144 ± 92.97[a]
IV	EPE (100)	1009 ± 186.71[a]	1134 ± 189.33[a]	1162 ± 84.99[a]	1202 ± 85.03[a]
V	EPE (300)	1237 ± 299.63[a]	992.2 ± 119.80[a]	1225 ± 110.71[a]	1185 ± 106.94[a]
VI	Etoricoxib (10)	1108 ± 305.50[a]	1128 ± 98.46[a]	1227 ± 98.53[a]	1270 ± 137.46[a]
Vertical spontaneous movements (Number of interruptions/5 min; mean ± SD)					
I	Naïve control	237.7 ± 55.76[a]	283.7 ± 67.27[a]	292.0 ± 53.87[a]	292.5 ± 39.22[a]
II	Vehicle control	241.8 ± 40.08[a]	120 ± 20.75[b]	103 ± 15.72[b]	103.5 ± 8.01[b]
III	EPE (30)	291.6 ± 64.70[a]	273.5 ± 43.92[a]	274.3 ± 34.20[a]	286.5 ± 20.77[a]
IV	EPE (100)	244.0 ± 17.19[a]	267.5 ± 58.99[a]	287.0 ± 26.75[a]	292.3 ± 17.45[a]
V	EPE (300)	265.5 ± 66.00[a]	285.7 ± 28.95[a]	290.5 ± 28.83[a]	291.5 ± 21.53[a]
VI	Etoricoxib (10)	250.3 ± 43.65[a]	262.5 ± 65.07[a]	277.5 ± 68.67[a]	301.7 ± 53.55[a]

(n = 6). All groups were compared with each other for significance; Values bearing no superscript in common differ significantly; $P<0.05$.

EPE 300 mg/kg and etoricoxib 10 mg/kg significantly increased paw withdrawal latency as compared to vehicle control rats on days 7 and 14. All doses of EPE employed in this study and etoricoxib 10 mg/kg significantly increased paw withdrawal on day 21 of observation as compared to vehicle-treated rats.

Cold hyperalgesia: Table I shows paw withdrawal latency (PWL) in seconds to noxious cold of different groups on days 0, 7, 14 and 21. PWL of right paw to noxious cold (4°C ±1.0) was similar in all groups at day 0 before MIA administration in right knee joint. On days 7 and 21, significant hyperalgesia to cold developed in vehicle-treated control group of rats as compared to naïve control but not on day 14 of MIA injection. Significant hyperalgesia was reduced on these days following EPE administration at 30, 100 and 300 mg/kg doses and also etoricoxib.

Spontaneous movements: Horizontal spontaneous movements (HSM) of all groups did not differ significantly on day 0. A significant decrease in movements was found in vehicle control on days 7, 14 and 21 whereas values were normal in other groups as compared with naïve control data (Table II). Vertical spontaneous movements (VSM) of all groups of rats did not differ significantly on day 0, but a significant decrease in VSM was found in vehicle control on days 0, 7, 14 and 21 whereas in other treatment groups, number of movements did not differ significantly compared with naïve control on these time points. This increase in movements in treatment groups corresponded to decrease in pain (hyperalgesia) (Table II).

Knee diameter: Knee diameters were taken on days 0 and 21 of all the groups. Data of knee diameter on these time points did not differ significantly on day 0 and further on day 21 indicating no inflammatory oedema at day 21 of the experiment (data not shown).

Histopathology: Cartilage of naive control rats showed intact superficial, mid and deep zones (from top to bottom of image). The chondrocytes were arranged in columns (Figs 1A and B). In MIA–treated vehicle control rats, in articular joint, articular cartilages of tibia as well as femur were completely absent in most the rats and replaced by fibrocartilage (Figs 1C and D). Bony tissue underneath fibrocartilage was found to be irregular in thickness and showed discontinuity at places. Proliferation of synovium was also observed

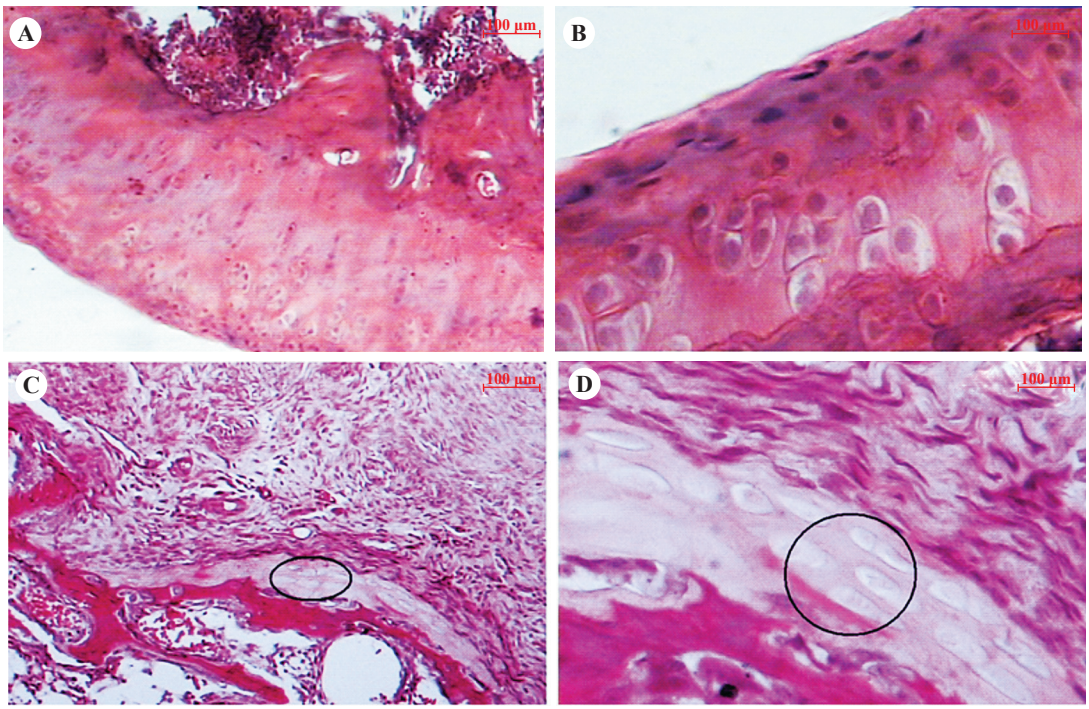

Fig. 1. Hematoxylin and eosin stained femoral articular cartilage of naïve control rats (**A**, 10x; **B**, 40x) showing the normal histology having smooth surface with intact superficial mid and deep zones, whereas vehicle-treated MIA rats cartilage (**C**, 10x; **D**, 40x) showing severely damaged cartilage with widespread cell necrosis, replaced with fibrocartilage (circle), irregularly arranged and clustering of chondrocytes.

and in some animals, it was so severe that complete synovial cavity was filled by fibrous tissue.

Histological changes observed in MIA-treated group of rats with lowest dose of EPE (30 mg/kg body weight) were identical to MIA- treated vehicle control group. Ameliorative effect was not seen in this group. Ameliorative effect was seen in Group IV where osteoarthritic rats were treated with EPE at a dose of 100 mg/kg body weight continuously for 21 days. A clear transition from fibrocartilage to cartilage formation was seen in this group (Figs 2A and B). In most of the animals complete articular cartilage was formed though it was hypocellular. EPE treatment (300 mg/kg body weight) distinctly prevented the damage to cartilage structure and reduced the cellular abnormalities. Articular cartilage of both femur and tibia in rats of group V were almost found to be normal with well-developed smooth surface, articular cartilage containing chondrocytes distributed individually or arranged in column (Figs 2C and D).

In rats treated with etoricoxib (group VI), articular surface (both femur and tibia) showed transition phase from fibrocartilage to cartilage formation. Degree of damage of articular cartilage was mild to moderate type. In some animals, fibrocartilage was found to be converted into cartilage but at some areas patches of fibrocartilage were present. Newly formed cartilage was acellular. Synovial membrane proliferation was present in articular cartilage. Protection was also seen in etoricoxib-treated group of rats.

Discussion

The present results showed development of secondary mechanical and heat hyperalgesia at all time points *i.e.* 7, 14 and 21 days post MIA administration while cold hyperalgesia developed at the end of study *i.e.* 21 days post MIA treatment. Little is known regarding the exact mechanisms responsible for MIA-induced joint pain but it may be related to early inflammatory reactions in the joint. The acute inflammatory response in the MIA model lasts approximately three days during which time trophic factors are likely to be released into the joint that upregulate pro-nociceptive receptor expression leading to a reduction in mechanosensory threshold[16]. According to Beyreuther and co workers[17] during the

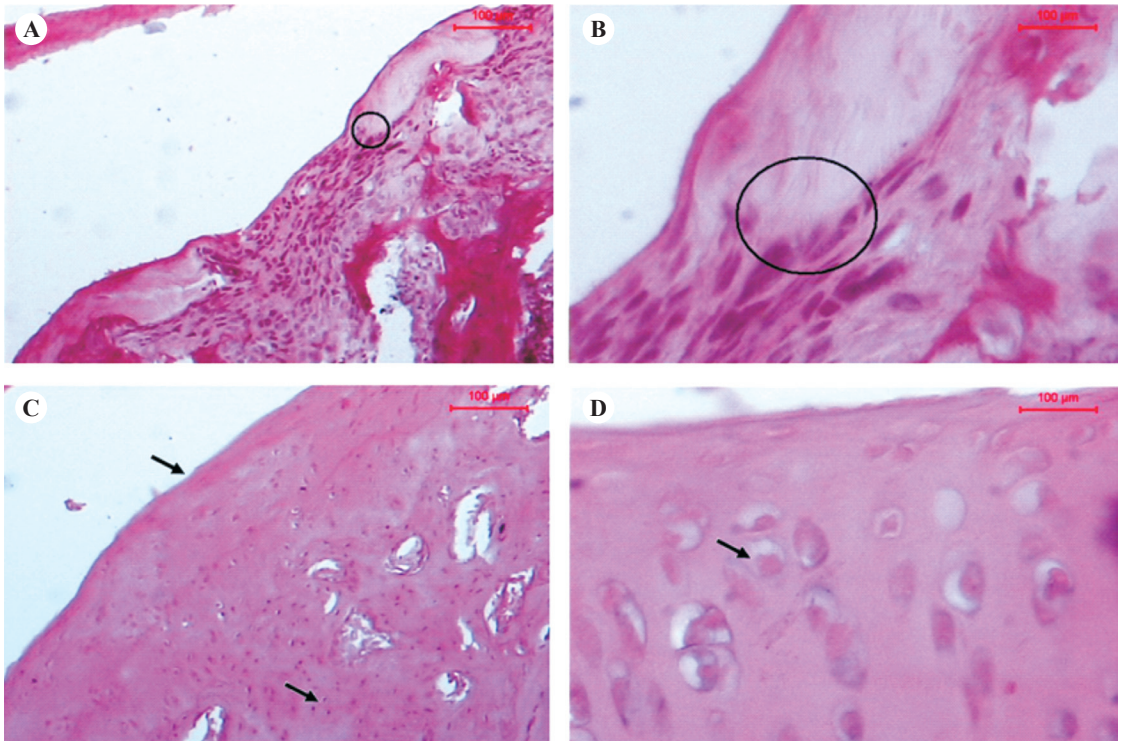

Fig. 2. Hematoxylin and eosin stained femoral articular cartilage of 100 mg/kg EPE treated rats showing transition of fibrocartilage (circle) to acellular cartilage (**A**, 10x; **B**, 40x) and 300 mg/kg EPE treated rats (**C**, 10x; **D**, 40x) showing reduced joint cartilage damage and cartilage surface with well developed superficial, mid and deep zones (arrows).

first weak, pain is induced mainly by inflammation in the iodoacetate model, but afterward inflammation plays a minor role in pain and it is more likely caused by biomechanical forces affecting articular cartilage and subchondral bone. Therefore, in the present study, three time points 7, 14 and 21 days were taken for pain determination to see the effect of EPE and etoricoxib on both inflammatory phase and neuropathic phase.

Our results demonstrated that etoricoxib mitigated secondary mechanical and heat hyperalgesia at all time points of observation and also mitigated cold hyperalgesia developed on day 21. Though data regarding use of etoricoxib in MIA- induced osteoarthritic pain model are not available, another coxib, celecoxib has been found to reduce mechanical secondary hyperalgesia in this model in early inflammatory phase and in the last phase of study, except for 18th and 23rd days of experiment[18]. *E. pursaetha* is a novel plant and no information is available regarding its mechanism of action in OA. EPE might have reduced production of inflammatory mediators responsible for peripheral and central pain sensitization and hyperalgesia in MIA model of OA.

The reduction of spontaneous activity by adjuvant (RSAA) as an objective and quantifiable behavioural model of inflammatory pain that can predict the analgesic activity of a variety of agents following single-dose administration of complete Freund's adjuvant had been noted[19]. Thus, RSAA model operationally defines analgesia as a drug-induced increase in spontaneous behaviour (vertical rearing in a novel environment) and is valuable as an objective measure of analgesic efficacy that is not dependent on an evoked stimulus response[20]. Such observations have prompted us to investigate the effect of EPE on spontaneous motor activity in MIA-induced osteoarthritis pain in this study Due to reduction of hyperalgesia by etoricoxib and EPE, horizontal and vertical spontaneous movements of rats were brought to normal in treatment group as compared to MIA-treated group suggesting lack of depressant effect of EPE on CNS and the anti-hyperalgesic effects of EPE were observed in the absence of any locomotor impairment. The ethanolic extract of *E. pursaetha* administered orally did not cause acute (24 h) and subacute (28 days) toxicities in the albino rats up to

2000 mg/ kg in an earlier study suggesting that it is quite safe for long term use[21].

In the present study, histological examination showed that intra-articular injection of MIA in rat knee joint resulted in cartilage matrix degradation and chondrocyte disorganisation. EPE at a dose of 300 mg/ kg mitigated MIA effect and at 100 mg/kg showed protective effect. Etoricoxib also exhibited protective effect.

The pathogenesis and source of pain in OA are uncertain. Although articular cartilage is primarily affected by the disease, it does not contain nerve fibres. Additional anatomic structures that have been proposed as potential sources of pain include the synovial capsule/membrane, menisci and subchondral bone[22]. Since subchondral bone is richly innervated, it is important to understand how the occurrence and progression of bone lesions relate to the onset of joint pain. In one study[13], evidence of subchondral bone involvement was apparent as early as seven days post-MIA. The changes are consistent with the initiation of bone remodelling and may have been induced by increased load in the subchondral bone due to the advanced loss of cartilage. Though we did not study histopathology of joint at seven days post MIA injection, it is hypothesized that pain and secondary hyperalgesia observed at this time point could be due to histopathological changes suggested by Guzman and coworkers[20]. Inhibition of osteoclastic activity when initiated early leads to improved efficacy[23]. Osteoclastic activity was decreased at day 21 with 300 mg/kg dose of EPE accounting for relief of pain at this time.

Results obtained in the present study revealed that total phenolic compounds in the ethanol extract of the stem of *E. pursaetha* were considerably high. Polyphenolic compounds are known to have antioxidant activity and it is likely that the activity of the extract is due to these compounds[24,25]. Oxidative stress is known to be associated with the development of OA and the beneficial therapeutic effects of antioxidants such as vitamin E and vitamin C have been demonstrated in animal studies of OA[26]. Total flavanoid, tannin and saponin contents were found to have anti-osteoarthritic effect in different studies[27-29] and our study findings suggested that EPE treatment led to a significant reduction in pain and structural changes in MIA-induced OA in rats.

In conclusion, the results demonstrated that EPE was not only able to mitigate pain and hyperalgesia but also inhibited MIA-induced cartilage degeneration *in vivo*. The disease modifying activity of EPE in osteoarthritis may provide a lead for a new treatment modality for osteoarthritis.

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References

1. Hayami T, Pickarski M, Zhuo Y, Wesolowski GA, Rodan GA, Duong LT. Characterization of articular cartilage and subchondral bone changes in the rat anterior cruciate ligament transection and meniscectomized models of osteoarthritis. *Bone* 2006; *38* : 234-43.
2. Rengel Y, Ospelt C, Gay S. Proteinases in the joint: clinical relevance of proteinases in joint destruction. *Arthritis Res Ther* 2007; *9* : 221-30.
3. Lories RJ. Changing the outcome of osteoarthritis: Still a challenge for cyclooxygenase 2 inhibitors. *Arthritis Rheum* 2012; *64* : 37-9.
4. Martel-Pelletier J, Pelletier JP. Effects of diacerein at the molecular level in the osteoarthritis disease process. *Ther Adv Musculoskelet Dis* 2010; *2* : 95-104.
5. Combe R, Bramwell S, Field MJ. The monosodium iodoacetate model of osteoarthritis: a model of chronic nociceptive pain in rats? *Neurosci Lett* 2004; *370* : 236-40.
6. Brenan JPM. Notes on Mimosoideae. *Kew Bull* 1955; *2* : 161-79.
7. Chopra RN, Chopra IC, Handa KL, Kapur LD. *Entada pursaetha*, In: *Chopra's Indigenous drug of India*, 1st ed, Academic Publisher, Kolkata; 2006 (reprint). p. 334-5.
8. Dhawan BN, Patnaik GK, Rastogi RP, Singh KK, Tandon JS. Screening of Indian plants for biological activity: Part VI. *Indian J Exp Biol* 1977; *15* : 208-19.
9. McDonald S, Prenzler PD, Autolovich M, Robards K. Phenolic content and antioxidant activity of olive extracts. *Food Chem* 2001; *73* : 73-84.
10. Polshettiwar SA, Ganjiwale RO, Wadher SJ, Yeol PG. Spectrophotometric estimation of total tannins in some ayurvedic eye drops. *Indian J Pharm Sci* 2007; *69* : 574-6.
11. Chang C, Yang M, Wen H, Chern J. Estimation of total flavonoids content in propolis by two complementary colorimetric methods. *J Food Drug Anal* 2002; *10* : 178-82.
12. Hiai S, Oura H, Nakajima T. Color reaction of some sapogenins and saponins with vanillin and sulfuric acid. *Planta Med* 1976; *29* : 116-22.
13. Vermeirsch H, Biermans R, Salmon PL, Meert TF. Evaluation of pain behavior and bone destruction in two arthritic models in guinea pig and rat. *Pharmacol Biochem Behav* 2007; *87* : 349-59.

14. More AS, Kumari RR, Gupta G, Lingaraju MC, Balaganur V, Pathak NN, *et al*. Effect of iNOS inhibitor S-methylisothiourea in monosodium iodoacetate-induced osteoathritic pain: implication for osteoarthritis therapy. *Pharmacol Biochem Behav* 2013; *103* :764-72.

15. Randall LO, Selitto J. A method for measurement of analgesic activity of inflamed tissue. *Arch Int Pharmacodyn Ther* 1957; *111* : 209-19.

16. Schuelert N, McDougall JJ. Grading of monosodium iodoacetate-induced osteoarthritis reveals a concentration-dependent sensitization of nociceptors in the knee joint of the rat. *Neurosci Lett* 2009; *13* : 184-8.

17. Beyreuther B, Callizot N, Stöhr T. Antinociceptive efficacy of lacosamide in the monosodium iodoacetate rat model for osteoarthritis pain. *Arthritis Res Ther* 2007; *9* : R14.

18. Ferland CE, Laverty S, Beaudry F, Vachon P. Gait analysis and pain response of two rodent models of osteoarthritis. *Pharmacol Biochem Behav* 2011; *97* : 603-10.

19. Matson DJ, Broom DC, Carson SR, Baldassari J, Kehne J, Cortright DN. Inflammation-induced reduction of spontaneous activity by adjuvant: A novel model to study the effect of analgesics in rats. *J Pharmacol Exp Ther* 2007; *320* : 194-201.

20. Guzman RE, Evans MG, Bove S, Morenko B, Kilgore K. Mono-iodoacetate-induced histologic changes in subchondral bone and articular cartilage of rat femorotibial joints: an animal model of osteoarthritis. *Toxicol Pathol* 2003; *31* : 619-24.

21. Suriyavadhana M, Pakutharivu T. Evaluation of acute and sub acute toxicity of ethanol extracts of *Entada pursaetha*, *Toddalia aculeata*, and *Ziziphus mauritiana*. *World J Life Sci Med Res* 2011; *1* : 43-7.

22. Creamer P, Hunt M, Dieppe P. Pain mechanisms in osteoarthritis of the knee: effect of intra-articular anesthetic. *J Rheumatol* 1966; *23* : 1031-6.

23. Strassle BW, Mark L, Leventhal L, Piesla MJ, Jian Li X, Kennedy JD, *et al*. Inhibition of osteoclasts prevents cartilage loss and pain in a rat model of degenerative joint disease. *Osteoarthr Cartil* 2010; *18* : 1319-28.

24. Okudu T, Yoshida T, Hatano T. Food phytochemicals for cancer prevention II. In: Ho C, Osawa T, Huang MT, Rosen RT. editors. *Chemistry and antioxidative effects of phenolic compounds from licorice, tea and Compositae and Labiateae herbs*. Washington, DC: American Chemical Society; 1994. p. 132-43.

25. Tepe B, Sokmen M, Akpulat HA, Sokmen A. Screening of the antioxidant potentials of six *Salvia* species from Turkey. *Food Chem* 2006; *95* : 200-4

26. Canter PH, Wider B, Ernst E. The antioxidant vitamins A, C, E and selenium in the treatment of arthritis: a systematic review of randomized clinical trials. *Rheumatology* 2007; *46* : 1223-33.

27. Farid R, Rezaieyazdi Z, Mirfeizi Z, Hatef MR, Mirheidari M, Mansouri H, *et al*. Oral intake of purple passion fruit peel extract reduces pain and stiffness and improves physical function in adult patients with knee osteoarthritis. *Nutr Res* 2010; *30* : 601-6.

28. Amarowicz R. Tannins: the new natural antioxidants? *Eur J Lipid Sci Technol* 2007; *109* : 549-51.

29. Wu W, Xu X, Dai Y, Xia L.Therapeutic effect of the saponin fraction from *Clematis chinensis* Osbeck roots on osteoarthritis induced by monosodium iodoacetate through protecting articular cartilage. *Phytother Res* 2010; *24* : 538-46.

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