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https://dx.doi.org/10.4314/nvj.v43i2.6 **ORIGINAL ARTICLE**

HONEY IMPROVES RADIOGRAPHIC FEATURES OF MONOSODIUM IODOACETATE-INDUCED STIFLE (KNEE) JOINT OSTEOARTHRITIS IN A RAT MODEL

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ABSTRACT

Osteoarthritis (OA) is the most common form of joint disease with over half of all people older than 65 years demonstrating radiographic changes of osteoarthritis in the knees. Honey is known to contain bioactive compounds that exert chondroprotective effects by counteracting the homeostatic dysregulation of the joint. However, its effect on the radiographic features of osteoarthritis has not been proven. This study was carried out to evaluate the effect of honey on radiographic features of monosodium iodoacetate (MIA)-induced knee osteoarthritis in female Wistar rats. Thirty female Wistar rats were randomly divided into five groups of six animals each. Animals in group one were healthy (control) rats, while animals in groups two to five were subjected to experimental osteoarthritis of the right knee joint induced by a single intra-articular injection of 1mg of MIA. The animals in groups two, three, four, and five were treated with normal saline (1ml/kg b. w.), arthocare (glucosamine/chondroitin sulfate 6.67/8.33mg/kg b. w.), low dose honey (250mg/kg b. w.) and high dose honey (1,000mg/kg b. w.) respectively. All treatments were administered orally once daily using an oral cannula for twenty-one days. All animals were subjected to radiographic assessment of the right knee joint before and after induction of OA, and after treatment. High and low-dose honey reversed the loss of joint space; sclerosis of the tibial plateau, medial, and lateral femoral condyles, when compared to the arthocare-treated and untreated groups. In conclusion, honey improved radiographic features of knee osteoarthritis in a rat model induced by monosodium iodoacetate.

Keywords: Honey, Monosodium iodoacetate, Osteoarthritis, Radiography, Rat.

INTRODUCTION

Osteoarthritis is "a disorder involving movable joints characterized by cell stress and extracellular matrix degradation, initiated by micro- and macro-injuries that activate mal-adaptive repair responses including pro-inflammatory pathways of innate immunity which manifests first, as a molecular derangement (abnormal joint tissue metabolism), followed by anatomic, and/or physiologic derangements (characterized by cartilage degradation, bone remodeling, osteophyte formation, joint inflammation and loss of normal joint function), that can culminate in illness" (OARSI). It is usually seen in weight-bearing joints (knees and hips), but can also affect other joints. It is associated with the degeneration of articular cartilage and changes to sub-chondral bone at the joint margins (Sarzi-Puttini *et al.,* 2005). A total of 130 million people worldwide, will suffer from osteoarthritis by the year 2050 (W.H.O.). Although a lot is known about the symptom of the disease, the pathophysiology behind the structural changes is complex. By understanding the mechanisms driving joint tissue destruction in osteoarthritis and identifying the key factors involved, new targets for therapy are emerging that will go beyond symptomatic relief to slowing or stopping the progression of osteoarthritis (Loeser *et al*., 2016). The monosodium iodoacetate (MIA)-induced osteoarthritis model is a chemical model regularly used to measure pain behavior and drug therapy to resolve pain in animals. This model may be more predictive of drug efficacy than other pain models used to test osteoarthritic drugs and it is generally used in mice and rats. The intra-articular MIA injection in the rat knee produces OA changes within 7 days post-MIA injection (Guzman *et al*., 2003; Janusz *et al*., 2002;). Noninvasive imaging modalities such as magnetic resonance imaging (MRI), ultrasonography (USG), and laboratory biomarkers are being utilized in clinical studies of osteoarthritis (Bruyere *et al.,* 2006; Kane *et al.,* 2003; Raynauld *et al*., 2003, 2004). However, based on regulatory standards, radiographic OA remains the most important means of evaluation for the natural progression of OA (Altman *et al.,* 1995; Altman & Gold 2007; Galli *et al.,* 2003; Gunther & Sun, 2009; Marshall *et al*., 2007; Menz *et al*., 2005, Menz *et al*., 2007; Nagaosa *et al*., 2000; Scott *et al*., 1993;). Several grading systems such as the Kellgren &Lawrence classification, Tonnis classification, Ahlback classification, and the Manchester scale, that have been established and evaluated in knee, hip, and foot OA studies are illustrated in atlases (Abadie *et al.,* 2004, Altman *et al.,* 1996; GREES, 1996; Rovati 2009). Skeletal changes thought to occur in response to this physical stress and other factors are most often assessed by radiography. These radiographic changes have been codified in the 5-points semi-quantitative Kellgren–Lawrence (KL) grading scale, which is widely used in OA clinical research (Croft 2005; Kellgren 1957; Paradowski *et al.,* 2014; Riddle *et al.,* 2013; Sheehy & Cooke, 2015). Honey has been shown to have anti-inflammatory and anti-nociceptive effects, as well as reverse disease progression in osteoarthritis (Jimoh-Abdulghaffaar & Owoyele, 2021). It has also been used as a therapeutic agent in the management of OA (Martinez-Amenta *et al.,* 2021). However, there is a paucity of research on its effect on the radiographic features of OA. Hence, the aim of this study was to investigate the effect of oral administration of honey on radiographic features of MIA-induced stifle (knee) OA in female Wistar rats.

METHODS

Ethical approval

This study was approved by the Ethical Review Committee of the University of Ilorin with the approval number: UERC/ASN/2019/1553.

Animal grouping and treatment

Thirty, 12-month-old, female, Wistar rats weighing between 200-250g were used for the study. The rats were obtained in Ilorin and subsequently housed in the animal house of the College of Health Sciences, University of Ilorin, where they were maintained under standard conditions with distilled water and rat pellet feed *ad libitum*. After an acclimatization period of two weeks, the rats were randomly distributed into five groups of six rats each: positive control group (healthy rats that received normal saline 1ml/kg b. w.), negative control/untreated group (rats induced with osteoarthritis that received normal saline 1ml/kg b. w.), reference group (rats induced with osteoarthritis and treated with arthocare: glucosamine/chondroitin sulfate 6.67/8.33mg/kg b. w.) (Fidson Healthcare Limited), low dose honey-treated group (rats induced with osteoarthritis and treated with honey 250mg/kg b. w.) and high dose honey-treated group (rats induced with osteoarthritis and treated with honey 1000mg/kg b. w.). Honey, arthocare, and normal saline were administered orally, once daily using an oral cannula for a period of twenty-one days.

Induction of osteoarthritis

The rats were anesthetized with an intraperitoneal injection of 75mg/kg b. w of ketamine hydrochloride (Aculife Healthcare Private Limited, India). A single intra-articular injection of 2mg/ml of MIA (Santa Cruz Biochemicals, USA) was prepared using normal saline as a vehicle and injected into the patella region of the right stifle (knee) joint of rats in groups two to five. Four weeks after the injection of MIA, loss of normal joint function (impaired movement) was observed in the rats. They were subjected to radiography and a diagnosis of OA was made based on the presence of standard radiographic signs such as joint inflammation, non-uniform loss of joint space, osteophyte formation, subchondral sclerosis, and cartilage degradation (OARSI).

Administration of honey and arthocare

Table honey was purchased from the University of Ilorin apiary and arthocare (Fidson Healthcare Limited) from a local pharmacy. The specified doses of honey (Owoyele *et al.*, 2011) and arthocare were administered to the respective groups via the oral route using an oral cannula. Animals were treated for a period of twenty-one days post-induction of OA.

Honey GCMS

The chemical analysis of the honey used for this study was carried out at the department of Chemical Engineering of the University of Ilorin using the gas chromatography-mass spectrophotometry (GCMS) method.

Table 1: Chemical Analysis of Honey using Gas Chromatography-mass Spectrophotometry (GCMS)

S/No.	RT	Area (%)	Compound	Ref	CAS	Qual
1	2.462	0.1	Ethoxy carbonyl isothiocyanate silane	13794	016182-04-0	56
			Trimethyl-1-cyclohexyl ethanol	799	000993-07-7	38
			Methyl ether-2-imidazolinedinone	200441	000365-12-8	25
2	2.800	2.33	1,3-dimethyl-3-isobutyldiaziridine	12381	000283-17-0	17
			Trimecaine	00713	000616-68-2	9
			3-imidazolinedinone	1608	000120-93-4	9
3	3.375	1.93	3-hexanone	3783	000589-38-8	59
			Cis-1-ethoxy-1-butene	38481	000139-43-8	45
			4-hexanone	3782	000589-38-8	45
4	3.713	0.29	1,3-dioxol-2-one	1601	000872-36-6	50
			1,3-dioxol-2-one	1602	000872-36-6	50
			2-imidazolinedinone	1608	000120-93-4	45
5	3.845	0.57	Furan-2,3-dihydro-4-methyl-2-butenal	1445	034314-83-5	53
			2-methyl-(e)	1441	000497-03-0	52
			2-pentenal-(e)	1405	001576-87-0	52
6	4.420	7.60	Ethyl beta-d-riboside pentanoic acid	443091	000126-95-4	9
			Butanoic acid	4426	000109-52-4	9
			3,3-dimethyl-methyl ester	13569	010250-48-3	9
7	5.008	17.55	1,4-cyclohexane diol, transpropanenitrile	8152	006995-79-5	28
			3-butoxy-1,3-benzodioxol-2-one	11830	006959-71-3	25
			Hexa-hydro cis	19504	019456-20-3	

8	5.909	17.79	1,3-butadiene-1-carboxylic acid	3111	000626-99-3	53
			2-furanmethanol	3079	000098-00-0	47
			Methylenecyclopropane carboxylic acid	3117	062266-36-8	47
9	6.822	8.10	Furan carboxaldehyde	5773	000620-02-0	58
			5-methyl-2-furan carboxaldehyde	5771	000620-02-0	58
			5-methyl-2-furan carboxaldehyde	5772	000620-02-0	52
10	7.035	5.22	1,1-cyclohexanedimethanol	20882	002658-60-8	53
			Furan-2,5-dimethyl	2802	000625-86-5	46
			Furan-2,5-dimethyl	2805	000625-86-5	46
11	7.698	0.53	Butanoic acid	72218	021282-97-3	53
			3-oxo-2- [(2-methyl-1-oxo-2-propenyl) oxy] ethyl ester cyclohexanone	6592	013368-65-5	53
			3-methyl, (R)-N- [4-bis (acetyl) aminobutyl] acetamide	722481	000378-73-6	17
12	8.511	0.59	Furan, 2,3,5-trimethyl-2-cyclopenten-1-one	5810	010504-04-8	43
			2.3-dimethyl-1-H-pyrazole	5862	001121-05-7	38
			1,3,5-trimethyl	5739	001072-91-9	35
13	8.986	1.83	1,2-cyclopentanedione, 3-methyl			91
			2-Cyclopenten-1-one, 2-hydroxy-3-methyl	6424	000080-71-7	91
			2-Cyclopenten-1-one, 2-hydroxy-3-methyl	6426	000080-71-7	91
14	9.631	1.31	Ethane, 1-bromo-2-fluoro-	10935	000762-49-29	
			4-morpholine acetonitrile	11068	005807-02-3	7
			3,8-nonadien-2-one, (E)	17538	055282-90-1	5

15	10.607	3.72	2,5-dimethyl-4-hydroxy-3(2H)-furanone	12136	003658-77-3	52
			Furan-2-methyl-5-(methylthio)-6-amino-	12169	013678-59-6	47
			1,3,5-triazine-2,4 (1H, 3H)-dione	11966	000645-93-2	47
16	11.720	1.38	2-cyclopenten-1-one, 3-ethyl-2-hydroxy	11198	02185-01-8	60
			2-cyclopenten-1-one, 3-ethyl-2-hydroxy	11199	02185-01-8	49
			3-H-pyrazol-3-one-2,4-dihydro-2,4,5-trimethyl	11083	017826-82-3	38
17	11.764	1.22	Indeno-[3a,4b]-oxiren-2-ol, octahydro-4a-methyl-5-[(tetrahydro-2H-pyran-2-yl) oxy]	117248	067920-65-4	45
			4-(3-methoxycarbonylpropyl)-4-butanolide	50783	100145-24-2	36
			2,4:3,5-dimethylene-1-iditol	66418	1000128-41-8	33
18	12.308	1.14	4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxyl-5-methyl	20638	028564-83-2	64
			4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxyl-6-methyl-	20639	028564-83-2	64
			4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxyl-6-methyl-	20640	028564-83-2	50
19	12.796	1.00	Stevioside	243519	000077-05-4	16
			1H-imidazole, 2,4,5- trimethyl-	5741	000822-90-2	14
			Methyl alpha d-rhamnopyranoside	44317	001128-40-1	12
20	13.046	0.58	O-trifluoro acetyl-neomenthol cyclohexane	103570	028587-52-2	53
			1-methyl-3-(1-methylethyldiene)	16992	013828-34-7	53

			Cyclohexanone-2-(1-methylethyldiene)	7610	013747-73-4	49
21	13.715	11.07	5-hydroxymethylfurfural	11111	000067-47-0	53
			2-thiophene-ethanol	12162	005402-55-1	38
			5-hydroxymethylfurfural	11110	000067-47-0	38
22	14.472	2.74	1,2-Benzenediol, 4-methyl-	10433	000452-86-8	91
			1,2-Benzenediol, 4-methyl-	10429	000452-86-8	87
			1,2-Benzenediol, 4-methyl-	10436	000452-86-8	87
23	14.841	1.58	1-(2-Hydroxy-ethyl)-4,6-dimethyl-	37156	014716-32-6	30
			1H-pyrimidin-2-one-malononitrile, o-chlorobenzyl-	52819	040915-55-7	27
			Benzene, (2-methylpentyl)	32252	039916-61-5	25
24	15.579	2.76	Tricyclo [6.3.0.0 (1,5)] undecane-4-one	54369	1000153-99-8	30
			5,9-dimethyl-1,4-benzendiol-2,6-dimethyl	17357	000654-42-2	22
			1,4-benzendiol- 2,5-dimethyl	17362	000615-90-7	22
25	16.042	1.38	Butanal-2-methyl propenamide	1744	000096-17-3	16
			N-(1,1-dimethyl)-2,2-dimethyl	29513	000686-96-4	12
			N, N'-bis (2-methyl-2-nitrosobutanone)	385509	034946-73-1	12
26	16.280	1.36	1,3-spiroheptadine dimer	48926	1000221-91-3	14
			Benzene nonyl-	64299	001081-77-2	14
			Benzene heptyl-	42586	001078-71-3	14
27	16.493	1.29	Methyl tetra decanoate	95862	000124-10-7	93
			Methyl tetra decanoate	95859	000124-10-7	93

			Methyl tetra decanoate	95862	000124-10-7	90
28	16.82	1.24	Stearic acid hydrazide	143051	004130-54-5	49
			11,13-dihydroxy-tetradec-5-enoic acid	120957	1000193-81-4	35
			Methyl ester-2-octene. 1 (methoxy)	3956	142509-32-8	27
29	17.688	1.40	Pentadecanoic acid-14-methyl, methyl ester	119423	005129-60-2	98
			Hexadecenoic acid	119400		
			Hexadecenoic acid	119405	000112-39-097	
30	18.094	0.08	Tetra decanoic acid-5,9,13-trimethyl-, methyl ester	131323	056196-55-5	35
			Methyl-2-O-methyl beta-1-arabinopyranside	44326	007381-11-5	27
			Heptadecanoic acid-15-methyl-methyl ester	143186	05483355-5	27

Radiographic examination

Radiographs were taken before the experimental induction of OA to ascertain the pre-experimental state of the stifle joints, as well as four weeks after induction of osteoarthritis to confirm successful induction of OA, and after treatment with honey and arthocare for a period of twenty-one days.

Animals were anesthetized with intra-peritoneal injection of 5mg/ml of 1% ketamine hydrochloride. Radiographs of right stifle joints were obtained with a mobile Allengers Mars 6R Veterinary x-ray apparatus (Allengers Medical Systems Limited, India) and film cassettes. The radiographs were obtained with exposure factors as follows: tube current of 10mA; tube voltage of 10-30 (too high for rats) kVp; an object-focus distance of 1m, a sufficient distance to make magnification negligible; and an exposure time of 10-100 milliseconds. The right stifle joint of each rat was x-rayed in 2 projections (craniocaudal and lateral) and in the same manner by the same radiographer. This was done before and after induction of osteoarthritis to be sure that the stifle joints were normal and induction of OA was successful, as well as at the end of treatment with honey and arthocare for a period of twenty-one days. The X-ray films were effectively processed in the darkroom and the radiographic images obtained were examined using a standard X-ray viewer and analyzed independently by two experienced radiologists

(without the knowledge of the joints and sub-grouping of the research animals) using masked subjective comparison using the Kellgren-Lawrence Grading Scale which grades OA as grade:
Grade 0: No pathological features
Grade 1: Doubtful narrowing of the joint space and possibly osteophyte lipping
Grade 2: Definite osteophytes and possibly narrowing of joint space.
Grade 3: Moderate multiple osteophytes, definite narrowing of joint space, some sclerosis, and possibly deformations of bone ends
Grade 4: Large osteophytes, marked narrowing of joint space, severe sclerosis, and definite deformity of bone ends.

RESULTS

Following masked, subjective comparison by two independent evaluators using the Kellgren-Lawrence grading scale, the pre-induction radiographs showed normal, healthy right stifle joints in all the animals across all the five groups (Grade 0). A representative radiograph is shown in Figure 1.

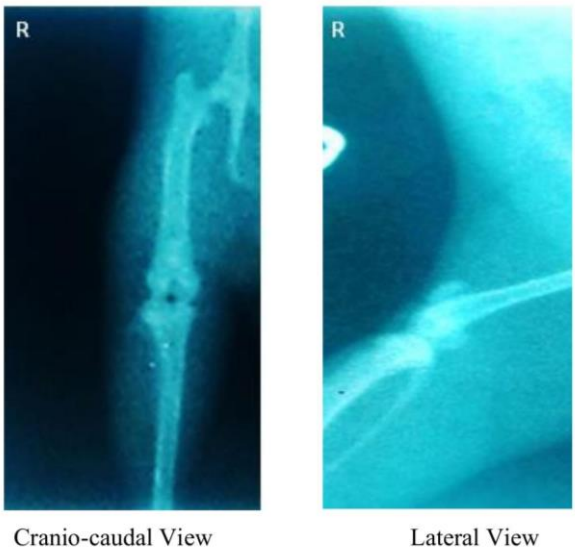

Figure 1: Pre-induction radiograph (normal stifle joint)
Showing even muscle mass, presence of menisci, normal joint space, smooth medial and lateral condyles in contact with the tibial plateau, normal patellofemoral groove, open tibial crest, and no osteophyte formation.

The post-induction radiographs showed features of OA (Grade 3). A representative radiograph is shown in Figure 2.

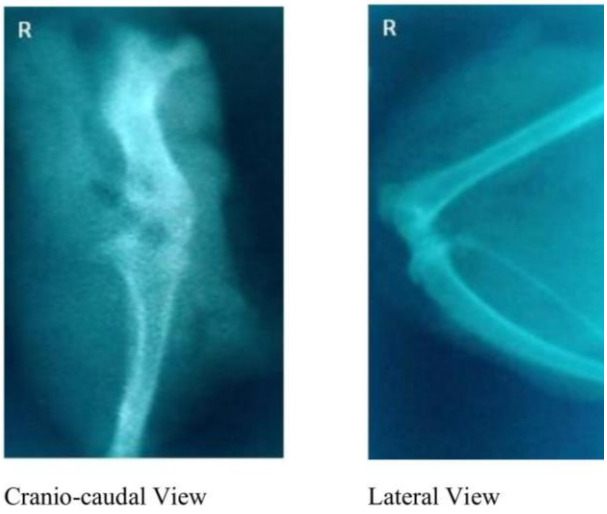

Figure 2: Post-induction radiograph (osteoarthritic stifle Joint)
Shows massive selerosis of the plateau, almost resulting in ankylosis, the closing of the tibial crest, articular osteophyte projection of the medial condyle, and selerosis of the lateral condyle.

The radiographs that were taken after treatment showed a marked improvement of the osteoarthritic features in the standard and test groups (Grade 1). This is shown in the representative radiograph in Figures 3, 4, and 5.

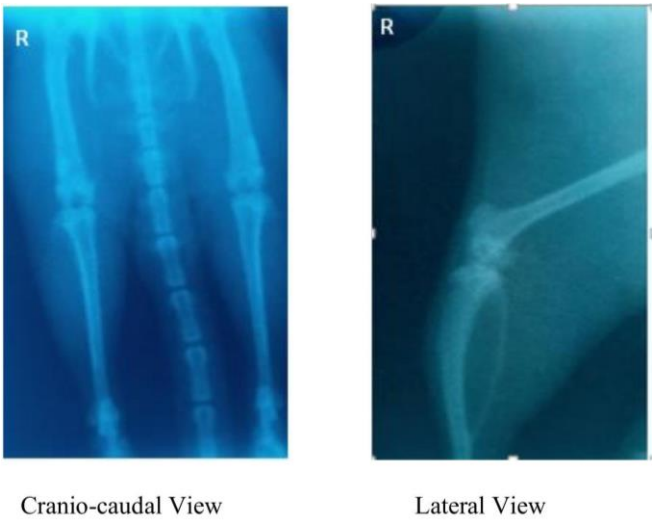

Figure 3: Post-treatment radiograph (arthocare)
Showing lytic lesions in the femur, marked selerosis of the medial condyle of the femur.

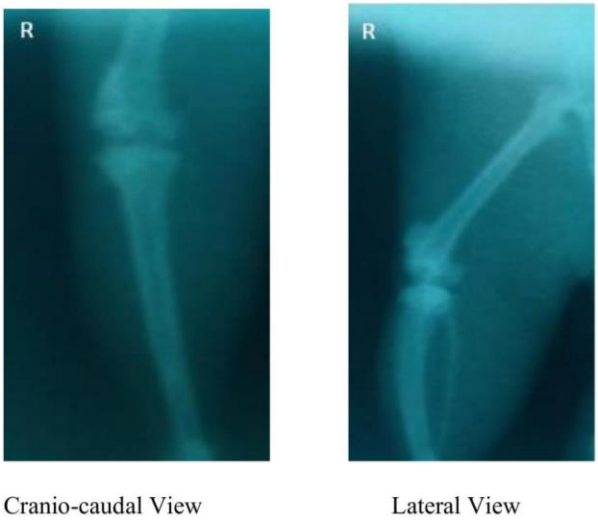

Figure 4: Post-treatment radiograph (low dose honey)
Showing a slight reduction in the selection of the tibial plateau, selerosis of the lateral condyle touching on the tibial plateau, and an almost closed femoro-patellar joint.

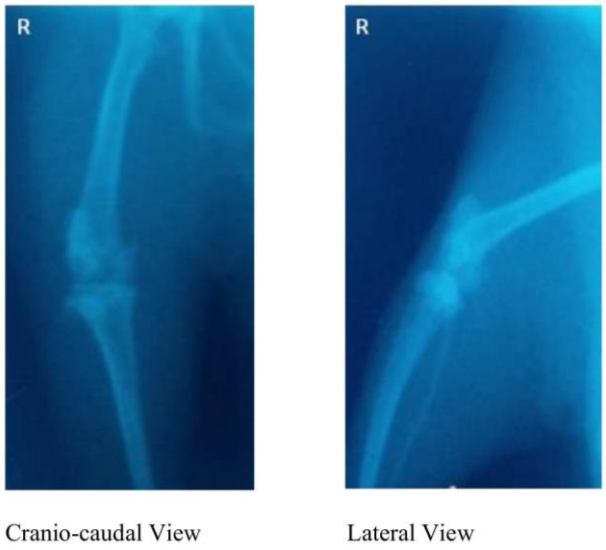

Figure 5: Post-treatment radiograph (high dose honey)
Showing a marked decrease in selerosis of the tibial plateau, mild selerosis of the medial and lateral femoral condyles, and minimally increased joint space.

DISCUSSION

The chemical model of induction of osteoarthritis using mono-sodium iodoacetate shows degeneration of chondrocytes, which are responsible for maintaining the integrity of the articular cartilage (Kobayashi *et al.,* 2003). Reparative inflammation also occurs, leading to bone sclerosis, pain, and a decrease in joint space, similar to human osteoarthritis (Janusz *et al.,* 2002). In addition to these, constant bone degeneration and osteophyte formation are seen in the radiographic images shown in this study. This model served as the basis for the experimental treatment of osteoarthritis in various ways (Cifuentes *et al.,* 2010; Albuquerque *et al.,* 2015; Maoo *et al.,* 2013). Honey bee venom has been shown to have anti-inflammatory, antioxidant, and immuno-modulatory effects in rheumatoid arthritis

(Kocigyit *et al.,* 2019). The anti-inflammatory effect of honey has also been reported by several researchers including (Hadagali *et al.,* 2014; Owoyele *et al.,* 2011; Bashkaran *et al.,* 2011; Hussein *et al.,* 2012). The findings of this study show that honey reverses adverse radiographic features of osteoarthritis caused by intra-articular injection of MIA. This is in keeping with reports from the study by Sahin which showed that honey improves radiographic features of fracture healing (Sahin *et al.,* 2018).

CONCLUSION

This study shows that honey improves radiographic features of mono-sodium iodoacetate-induced stifle (knee) osteoarthritis in Wistar rats.

DECLARATION OF INTEREST

The authors of this work declare that they do not have conflicting interests in carrying out the study.

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