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Original Article

Honey reverses disease progression, has anti-nociceptive and anti-inflammatory effects in a rat model of knee osteoarthritis induced by monosodium iodoacetate

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SUMMARY

Background: Clinically, osteoarthritis manifests as joint pain with concomitant loss of joint function, which may ultimately result in a substantially reduced quality of life for the patient. Although, a lot is known about the symptom of the disease, the pathophysiology behind the structural changes is complex and poorly understood. 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.
Aim: Thus, the aim of this study was to evaluate the effect of honey on disease progression, pain perception and inflammation in monosodium iodoacetate (MIA)-induced knee osteoarthritis in female Wistar rats.
Methods: Thirty, twelve-month old female Wistar rats, weighing between 200 g & 250 g, were randomly divided into five groups of six animals each. Animals in group one were not induced and served as the control, while animals in groups two to five were injected with monosodium iodoacetate in the right knee. In addition, animals in group two received normal saline (1 ml/kg b.w.), group three received arthocare (glucosamine/chondroitin sulphate 6.67/8.33 mg/kg b.w.), group four received low dose honey (250 mg/kg b.w.) while group five received high dose honey (1,000 mg/kg b.w.) and were treated for twenty one days. All animals were subjected to assessment of tactile allodynia (von Frey test), acute inflammation (knee edema), and serum biomarkers: tumour necrosis factor-alpha (TNF-α), vascular endothelial growth

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factor (VEGF), prostaglandin E_2 (PG E_2) & cartilage oligomeric matrix protein (COMP) as well as histo-pathological assessment of the right knee joint.
Results: Honey (at high and low doses), significantly ($p < 0.05$) reduced tactile allodynia on von Frey test (60 ± 20g) in animals subjected to experimental knee osteoarthritis induced by MIA. Knee edema was also significantly ($p < .05$) reduced by both high and low doses of honey. Low dose honey significantly ($p < .05$) reduced he serum levels of TNF-α (61.5 ± 22 pg/ml), VEGF (31 ± 6.1 pg/ml) and COMP (41 ± 14 ng/ml) but, had no effect on the serum level of PG E_2. High dose honey on the other hand, only significantly reduced the serum level of TNF-α (87 ± 22 pg/ml) but, had no effect on the serum levels of VEGF, COMP and PG E_2. However, the administration of honey did not show any significant effect on histo-pathological features of the induced osteoarthritis.
Conclusion: Honey reversed disease progression and reduced pain perception as well as inflammation in MIA-induced knee osteoarthritis in female Wistar rats. However, honey had no effect on the histo-pathology of the knee joint.

1. Introduction

According to the Osteoarthritis Research Society International [1], osteoarthritis is “a disorder involving movable joints characterized by cell stress and extracellular matrix degradation initiated by micro- and macro-injury that activates maladaptive 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”.

It is usually seen in weight-bearing joints (knees and hips). It is associated with degeneration of articular cartilage and changes to subchondral bone at the joint margins [2,3]. In 2010, the Global Burden of Disease Study reported that the burden of musculoskeletal disorders is much larger than estimated in previous assessments and accounts for 6.8% of Disability-Adjusted Life Years (DALYs) worldwide. The World Health Organization (W.H.O.) estimates that by 2050, a total of 130million people worldwide, will suffer from osteoarthritis.

Although, a lot is known about the symptoms of the disease, the pathophysiology behind the structural changes is complex and poorly understood. Currently, there are no commercially available pharmacologic approaches clinically proven to alter the progression of the disease [4]. 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 [5].

Monosodium iodoacetate-induced osteoarthritis model is a chemical model of osteoarthritis in rodents. The model may be more predictive of drug efficacy than other OA models used to test osteoarthritis drugs and it is generally used in mice and rats. The intra-articular MIA injection in the rat knee produced OA changes within 7 days post-MIA injection [6,7].

Honey is gaining acceptance as an agent for the treatment of ulcers, bed sores and other skin infections resulting from burns and wounds [8,9]. It has also being shown that honey has antinociceptive as well as anti-inflammatory effects [10]. Providing scientific evidence for the role of honey in altering the disease progression as well as providing pain relief in osteoarthritis will help

substantiate the claims of alternative medicine of honey as a cheaper and effective alternative to known pharmacologic agents in the treatment of osteoarthritis.

The aim of this study was to determine the effect of honey on the severity of chronic pain perception in osteoarthritic female Wistar rats, while the objectives were to assess the effect of honey on pain perception in control and osteoarthritic female Wistar rats; expression of serum pain/osteoarthritis biomarkers in control and osteoarthritic female Wistar rats; and histo-pathological features in control and osteoarthritic female Wistar rats.

2. Materials and methods

2.1. Experimental animals

Thirty twelve month-old female Wistar rats weighing between 200 and 250 g obtained from Ilorin were used for the study.

2.2. Housing and husbandry

The rats were subsequently housed in the animal house of the Faculty of Basic Medical Sciences, University of Ilorin, where they were maintained in plastic cages with net covers under standard conditions with distilled water and rat pellet ad libitum. The animals were allowed to acclimatize for a period of two weeks.

2.3. Sample size

A total of thirty animals were used for this study.

2.4. Allocating animals into experimental groups

They were randomly distributed into five groups of six rats each. Animals in group one were not induced and served as the control, while animals in groups two to five were injected with monosodium iodoacetate in the right knee. In addition, animals in group two received normal saline (1 ml/kg b.w.), group three received arthocare (glucosamine/chondroitin sulphate 6.67/8.33 mg/kg b.w.), group four received low dose honey (250 mg/kg b.w.) while group five received high dose honey (1,000 mg/kg b.w.) and were treated for twenty one days.

2.5. Ethical statement

This study was carried out based on the institutional guidelines of the University of Ilorin and an ethical approval was obtained from the University's ethical review committee with the number: UERC/ASN/2019/1553.

2.6. Study design

There were three control groups: positive control (healthy, untreated), negative control (osteoarthritic, normal saline-treated) and reference control [osteoarthritic, arthocare (glucosamine/chondroitin sulphate)-treated] groups; as well as two experimental groups: low dose honey-treated and high dose honey-treated groups. Three control groups were chosen because the study wanted to compare the effect of honey against osteoarthritic rats treated with standard drugs and those untreated at all, as well as against healthy rats to see if it offered any protective effects even in healthy state. Two experimental groups were also chosen to determine which dose of honey offered the best effects. Animals were randomly allocated into these groups and maintained in plastic cages with net covers under standard conditions with distilled water and rat pellet *ad libitum*.

3. Experimental procedures

3.1. Induction of osteoarthritis

The rats were anaesthetized with intra-peritoneal injection of 5 mg/ml of ketamine hydrochloride (Aculife Healthcare Private Limited, India). The joint space was identified by flexion of the right knee at 900, patella ligament was palpated below the patella and injection was made into this region. A single intra-articular injection of 2 mg/ml of monosodium iodoacetate (Santa Cruz Biochemicals, USA) was prepared using normal saline as vehicle and injected into the patella region. All rats except those in the positive control group were injected.

3.2. Administration of honey and arthocare (glucosamine/chondroitin sulphate)

Honey was purchased from the University of Ilorin apiary and arthocare from Health Plus pharmacy, Ilorin. Required doses of honey- 250 mg/kg b.w. and 1,000 mg/kg b.w. [11]; and arthocare (glucosamine/chondroitin sulphate) - 6.67/8.33 mg/kg b.w. (based on total daily dose in humans), were administered to the respective groups via the oral route using oral cannula. Animals were treated for a period of twenty one days after which they were sacrificed.

Chronic pain was evaluated in the animals using mechanical sensitivity (paw withdrawal) test as described earlier [12]. Mechanical withdrawal threshold of the hind paw was tested using a von Frey monofilament hair (VFH) via the ascending stimulus method. Monofilaments of VFHs with increasing bending forces from 2g to 100g, beginning with the lowest force, then gradually ascending to the next higher force, were applied at the center of the plantar region of the ipsilateral hind paw for 5 s till a slight bending was observed. The force of the von Frey filament that elicits this positive response is designated as the mechanical withdrawal threshold. This was done before induction and weekly after the induction of OA, until animals were euthanized.

3.3. Measurement of knee diameter

Animals were restrained on their backs to a board. Right hind paw was extended, a thread was wound round the knee and edges were marked. The marked edges were then placed on a ruler and reading was taken [13]. Procedure was repeated three times and an average of the three values was taken as the diameter. This was done before induction, post-induction and before sacrifice. Euthanasia was performed by giving an overdose (1 ml) of the anesthetic agent-ketamine hydrochloride. The animals were then confirmed dead by bilateral thoracotomy as described by the Institutional Animal Care and Use Committee.

3.4. Collection of knee joint

After euthanasia, each animal was placed in dorsal recumbency. Using forceps, the skin and subcutaneous tissue were freed from patellar tendon and the anterior aspect of the joint was exposed. The patellar tendon and patella were raised proximally from the lower portion of its insertion on the tibial tuberosity. An arthrotomy was then performed with a scalpel and that allowed the identification of the synovial membrane following en-bloc resection. The right knee joint was then excised from animals in each group.

3.5. Histo-pathological examination

The right knees of representative animals from each of the groups were carefully excised and fixed in 10% neutral-buffered formalin for 24 h. They were then decalcified for five days using decalcifying solution (7% w/v $AlCl_{36}H_2O$, 5% formic acid and 8.5% hydrochloric acid). They were sequentially dehydrated in 70%, 80%, 90%, and 100% ethanol. Finally, sections were cleared in xylene. The tissues were infiltrated with molten wax, embedded and thin sections were cut at 3 μm. The slides were

stained with hematoxylin and eosin (H&E) stain. A light microscope was used to review and examine the articular joint.

3.6. Experimental outcomes

Behavioral changes such as assessment of tactile allodynia using von Frey hair monofilament was carried out weekly till animals were sacrificed. Knee diameter of all the animals was also measured before induction, after induction and after treatment. Molecular biomarkers such as tumour necrosis factor-alpha (TNF-α), vascular endothelial growth factor (VEGF), prostaglandin E_2 (PG E_2) & cartilage oligomeric matrix protein (COMP) were assayed from the serum following sacrifice. After sacrifice, histo-pathological assessment of the right knee joint of one animal from each group was carried out.

3.7. Statistical methods

All data were expressed as the Mean ± S. E. M. The effects of the varied intervention of each of group were tested for homogeneity using one-way analysis of variance (ANOVA) through GraphPad prism version 5.01software. The *post-hoc* test used was Tukey. The level of significance was set at $p < 0.05$.

4. Results

4.1. Effect of honey on knee edema

The effect of the reference drug-arthocare (glucosamine/chondroitin sulphate) and honey on knee edema in monosodium iodo-acetate-induced osteoarthritis in rats is shown in Fig. 1a and b respectively. The results show that honey and arthocare significantly ($p < 0.05$) reduced the knee circumference of osteoarthritic rats at the end of the treatment on day 29 compared with day 8.

4.2. Effect of honey on tactile allodynia

von Frey test was carried out to assess pain perception to mechanical stimulus (touch), which will ordinarily not cause pain (tactile allodynia) among all the groups. The effect of the reference drug-

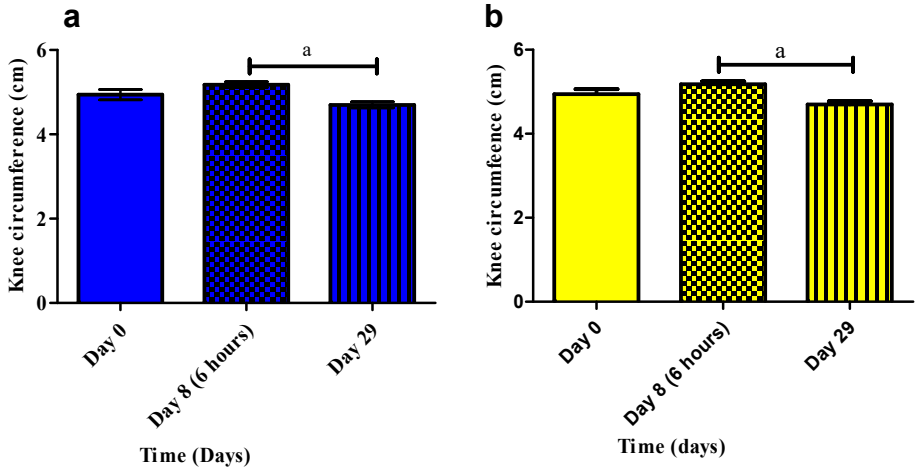

Fig. 1. a: Knee circumference in arthocare (glucosamine/chondroitin sulphate)-treated rats. Data are expressed as the mean ± SEM (n = 6 per group) assessed by one-way ANOVA, followed by Tukeys *post-hoc* analysis. **[a]p<0.05 is significant, compared with day 8.** **b**: Knee circumference in low dose honey-treated rats. Data are expressed as the mean ± SEM (n = 6 per group) assessed by one-way ANOVA, followed by Tukey's *post-hoc* analysis. **[a]p<0.05 is significant, compared with day 8.**

arthocare (glucosamine/chondroitin sulphate) and honey on tactile allodynia in monosodium iodo-acetate-induced osteoarthritis in rats is shown in Fig. 2a–e. The results show that honey and arthocare significantly ($p < 0.05$) reduced the tactile allodynia of osteoarthritic rats during treatment on days 15, 22 and 29, compared with days 0 and 8.

5. Serum biomarkers

5.1. Tumour necrosis factor-alpha (TNF-α)

The effect of oral administration of honey at low and high doses on serum TNF-α in monosodium iodo-acetate-induced osteoarthritis in rats is shown in is shown in Fig. 3a. The results show that honey at low (62 ± 14 pg/ml) and high (87 ± 22 pg/ml) doses significantly ($p < 0.05$) reduced the serum TNF-α level in osteoarthritic rats compared with the normal saline-treated group.

5.2. Vascular endothelial growth factor (VEGF)

The effect of oral administration of honey at low dose on serum VEGF in monosodium iodo-acetate-induced osteoarthritis in rats is shown in is shown in Fig. 3b. The results show that honey at low dose significantly ($p < 0.05$) reduced the serum VEGF level in osteoarthritic rats compared with healthy and normal saline-treated (31 ± 6.1 pg/ml) rats.

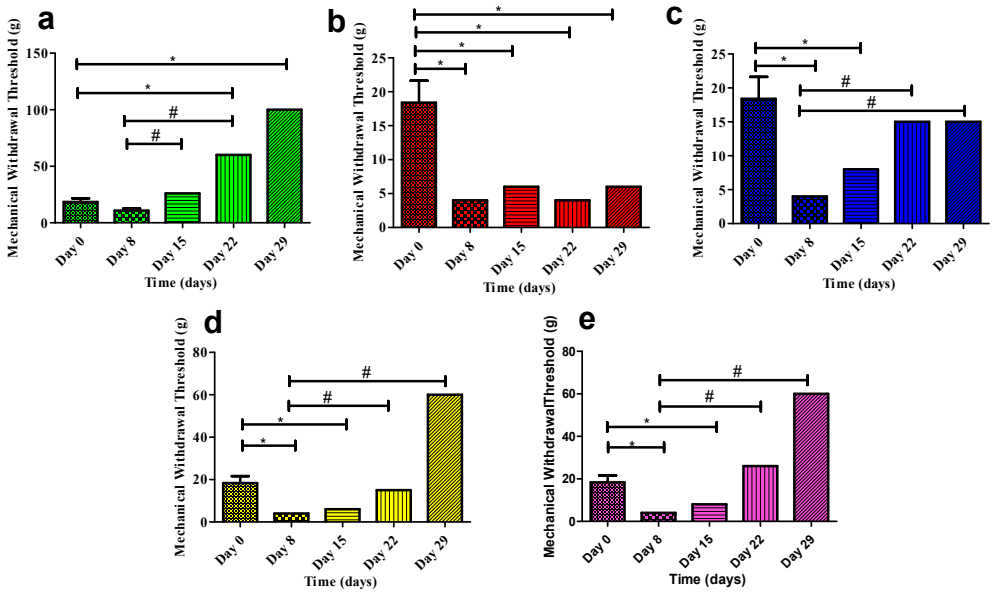

Fig. 2. **a**: Tactile allodynia in healthy rats. Data are expressed as the mean ± SEM (n = 6 per group) assessed by one-way ANOVA, followed by Tukey's *post-hoc* analysis. *$p < 0.05$, #$p < 0.05$ are significant, compared with days 0 and 8 respectively. **b**: Tactile allodynia in normal saline-treated rats. Data are expressed as the mean ± SEM (n = 6 per group) assessed by one-way ANOVA, followed by Tukey's post-*hoc* analysis. ***p<0.05 is significant, compared with day 0. c**: Tactile allodynia in arthocare-treated rats. Data are expressed as the mean ± SEM (n = 6 per group) assessed by one-way ANOVA, followed by Tukey's *post-hoc* analysis. ***p<0.05, #p<0.05 are significant compared with days 0, and 8 respectively. d**: Tactile allodynia in low dose honey-treated rats. Data are expressed as the mean ± SEM (n = 6 per group) assessed by one-way ANOVA, followed by Tukey's *post-hoc* analysis. ***p<0.05, #p<0.05 are significant compared with days 0, and 8 respectively. e**: Tactile allodynia in high dose honey-treated rats. Data are expressed as the mean ± SEM (n = 6 per group) assessed by one-way ANOVA, followed by Tukey's *post-hoc* analysis. ***p<0.05, #p<0.05 are significant compared with days 0, and 8 respectively**.

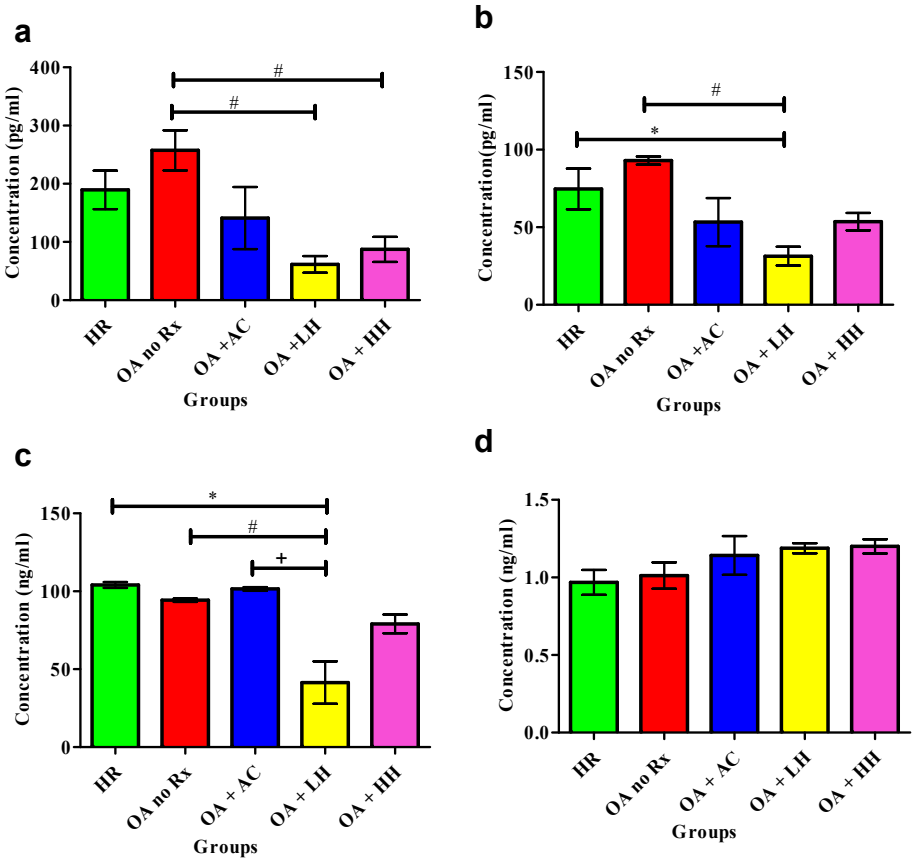

Fig. 3. **a**:Serum TNF-α in all groups after treatment. Data are expressed as the mean ± SEM (n = 6 per group) assessed by two-way ANOVA, followed by Tukey's *post-hoc* analysis. **$^{\#}$p<0.05 is significant, compared with the normal saline-treated group.** HR = Healthy rats, OA no RX = untreated osteoarthritic rats, OA+AC = Osteoarthritic rats that were treated with arthocare, OA+LH = Osteoarthritic rats that were treated with low dose of honey, OA+HH = Osteoarthritic rats that were treated with high dose of honey. **b**: Serum VEGF in all groups after treatment. Data are expressed as the mean ± SEM (n = 6 per group) assessed by one-way ANOVA, followed by Tukey's *post-hoc* analysis. ***p<0.05 and $^{\#}$p<0.05 are significant, compared with the healthy and osteoarthritic, normal saline-treated rats respectively.** HR = Healthy rats, OA no RX = untreated osteoarthritic rats, OA+AC = Osteoarthritic animals that were treated with arthocare, OA+LH = Osteoarthritic animals that were treated with low dose of honey, OA+HH = Osteoarthritic animals that were treated with high dose of honey. **c**: Serum COMP in all groups after treatment. Data are expressed as the mean ± SEM (n = 6 per group) assessed by one-way ANOVA, followed by Tukey's *post-hoc* analysis. *p < 0.05, $^{\#}$p < 0.05, $^{+}$p < 0.05 are significant, compared with the healthy, normal saline-treated, and arthocare-treated (41 ± 14 ng/ml) rats respectively. HR = Healthy rats, OA no RX = untreated osteoarthritic rats, OA+AC = Osteoarthritic rats that were treated with arthocare, OA+LH = Osteoarthritic rats that were treated with low dose of honey, OA+HH = Osteoarthritic rats that were treated with high dose of honey. **d**: Serum PG E2 in all groups after treatment. Data are expressed as the mean ± SEM (n = 6 per group) assessed by two-way ANOVA, followed by Tukey's post-hoc analysis. There is no significant in the serum PG E2 across all the groups. HR = Healthy rats, OA no RX = untreated osteoarthritic rats, OA+AC = Osteoarthritic rats that were treated with arthocare, OA+LH = Osteoarthritic rars that were treated with low dose of honey, OA+HH = Osteoarthritic rats that were treated with high dose of honey.

5.3. Cartilage oligomeric matrix protein (COMP)

The effect of oral administration of honey at low dose on serum COMP in monosodium iodoacetate-induced osteoarthritis in rats is shown in is shown in Fig. 3b. The results show that honey at low dose significantly ($p < 0.05$) reduced the serum COMP level in osteoarthritic rats compared with healthy, normal saline-treated and arthocare-treated rats.

5.4. Prostaglandin E_2 (PG E_2)

The effect of oral administration of honey at low and high doses on serum PG E_2 in monosodium iodo-acetate-induced osteoarthritis in rats is shown in is shown in Fig. 3d. The results show that honey at low and high doses had no significant effect on the serum level of PG E_2 in osteoarthritic rats compared with the other groups.

5.5. Histo-pathological findings

The effect of oral administration of honey and arthocare on histopathologic features in monosodium iodo-acetate-induced osteoarthritis in rats is shown in Fig. 4a–e. The results show that honey and arthocare had no significant effect on histopathologic features in rats across all the groups.

6. Discussion

This study showed the effect of honey on disease progression, inflammation and pain perception in MIA-induced osteoarthritic rats. Edema/tumour (swelling) is one of the cardinal features of inflammation; others being rubor/erythema (redness), dolor (pain), calor (heat) and loss of function. Knee swelling following the induction of OA with MIA and the effect of honey on it was assessed in this study. It was observed from this study that oral administration of low but, not high dose honey significantly reduced knee edema post-treatment compared with post-induction of osteoarthritis. Kil-Joon Bae et al., also reported a decrease in paw edema following treatment with chondroT in a MIA-induced rat model of knee osteoarthritis [14]. Owoyele et al., also reported a reduction in paw size in formaldehyde-induced arthritis in rats treated with honey [15]. Similar findings were also reported by Saba et al., [16], who found that oral administration of Gelam honey significantly reduced paw edema in rats [16].

Neuropathic pain has been known to be associated with various symptoms such as tactile allodynia, hyperalgesia and hyperpathia [17]. In this study, the injection of MIA induced tactile allodynia in animals, as demonstrated by a significant ($p < 0.05$) decrease in the nociceptive paw withdrawal threshold of osteoarthritic animals. However, this was reversed by the oral administration of honey at both low and high doses in this study. Orita et al., also reported that paw withdrawal threshold was significantly decreased beginning on post-injection day 4 in the MIA-treated ipsilateral limbs ($P < 0.01$), although it did not significantly changed from day 7 through the end of the experimental period [18]. Li et al., reported a reversal of mechanical hyperalgesia especially on day 28 in rats with MIA-induced OA treated with early laser moxibustion [19].

Tumor necrosis factor-alpha (TNF-α) is a pro-inflammatory cytokine and a major mediator in inflammatory responses, inducing innate immune responses by activating T cells and macrophages. It stimulates the secretion of other inflammatory cytokines [20,21]. In this study, both low and high doses of honey significantly reduced the serum level of TNF-α compared to the untreated group. Studies have shown that honey reduces the release of nitrous oxide, histamines, and cytokines such as tumor necrosis factor-alpha (TNF-α), which may reduce inflammation and pain [22]. Saba et al., also reported a decrease in the serum level of TNF-α in rats pre-treated with honey [16]. Kil-Joon Bae et al., reported a decrease in the serum level of TNF-α following treatment with chondroT in a MIA-induced rat model of knee osteoarthritis [14].

Vascular endothelial growth factor (VEGF) is involved in OA specific pathologies including cartilage degeneration, osteophyte formation, subchondral bone cysts and sclerosis, synovitis, and pain. Increased VEGF levels are associated with OA progression. This study showed that low (but, not high) dose honey significantly reduced the serum level of VEGF compared to the control and untreated groups. This shows that low dose honey is effective in reversing the disease progression in OA. Other studies have shown that inhibition of VEGF signaling pathways and of angiogenesis is a promising approach in recent pre-clinical studies, demonstrating reduced destruction of joints and of associated pain in OA [23].

Cartilage oligomeric matrix protein (COMP) is also known as thrombospondin 5. It is a 524 kDa homopentameric, non-collagenous, extracellular matrix glycoprotein member of the thrombospondin

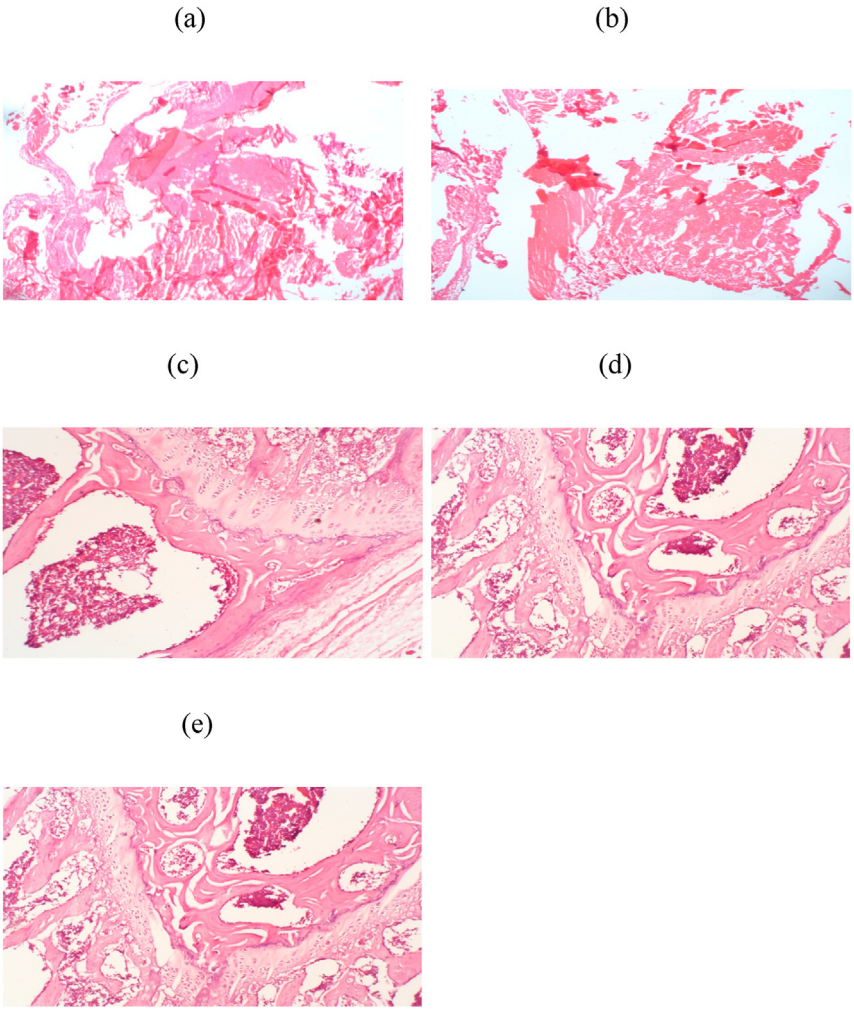

Fig. 4. Right knee sections with hematoxylin and eosin (H &E) stain. It shows no features of osteoarthritis in the excised right knee joints of the representative rats from all the groups. a = healthy rats, b = osteoarthritic, untreated rats, c = osteoarthritic rats treated with arthocare, d = osteoarthritic rats treated with low dose honey and e = osteoarthritic rats treated with high dose honey.

family of calcium-binding proteins [24]. It is expressed primarily in cartilage but, has also been identified in ligaments, meniscus, tendons, synovium, osteoblasts and vascular smooth muscle [25,26]. In various studies, COMP has shown promise as a diagnostic and prognostic indicator and as a marker of the disease severity and the effect of treatment [24]. Serum cartilage oligomeric matrix protein levels are higher in aggressive cases of arthritis and levels are used to predict future disease progression [27]. In this study, the serum level of COMP was also reduced in the group treated with low dose honey, compared to untreated and arthocare-treated groups. However, treatment with high dose honey had no effect on the serum level of COMP. In patients with established knee OA, the change in joint space width over 3 years, summed for both knees, correlated positively with serum COMP levels [24]. In rats with collagen-induced arthritis treated with corticosteroid therapy, serum COMP levels remained stable compared to increases in COMP over time in placebo-treated rats [28].

PG E_2 is a very important mediator of all types of inflammation and is responsible for increased prostaglandin production in inflamed tissue [29,30]. In this present study, it was found that honey at

both high and low doses, had no effect on the serum level of PG E_2. This is in contrast to the findings of an in vivo study which reported that Gelam honey and its extract have anti-inflammatory effects by reducing the inflammatory mediators such as PG E_2 in rat paw tissue [31]. Also, Saba et al., found that Gelam honey significantly reduced the production of serum PG E_2 [16]. This suggests that the anti-inflammatory effect of honey as observed in this study is not mediated by the inhibition of cyclo-oxygenase-2 (COX-2), the enzyme which catalyzes the conversion of arachidonic acid to prostaglandins, especially PG E_2. However, Kil-Joon Bae et al., reported a decrease in the serum level of PG E_2 following treatment with chondroT in a MIA-induced rat model of knee osteoarthritis [14].

Udo et al., reported that MIA-induced arthritis is dose and time-dependent and that at high doses of 0.5 mg and 1 mg, subchondral erosion only began at four weeks and thereafter. In this study, there were no histo-pathological abnormalities observed in all the groups [32]. This is similar to the findings of Bove et al., which also reported that there was absence of morphological changes in the knee in rats injected with 1 mg of MIA after seven days [4] as well as Ferreira-Gomes et al., who also reported that 1 mg (and 2 mg) of MIA did not induce bone destruction at four weeks of induction [33]. Although, similar dose of MIA (1 mg) was used in inducing OA in the same species (Wistar) of rats in these studies, no histo-pathological abnormalities were seen at four weeks. Also, Takahashi et al., reported fibrillation and fissuring in the patella and femur at four weeks, as well as erosion, denudation and replacement of articular cartilage with fibrous tissues at eight and twelve weeks, in rats with MIA-induced OA using 1 mg [34]. This suggests that longer duration should be used to observe histo-pathological changes in the knee of rats injected with 1 mg of MIA to induce OA.

The findings of this study suggest that honey improves tactile allodynia, knee edema as well as reduces the serum levels of tumour necrosis factor, vascular endothelial growth factor and cartilage oligomeric matrix protein and these could be replicable in human studies.

7. Conclusion

Low dose honey is more effective than high dose honey in reversing disease progression and producing anti-inflammatory as well as anti-nociceptive effects in MIA-induced osteoarthritis in female Wistar rats. Therefore, it is strongly recommended that a clinical trial on a large number of subjects should be carried out for the inclusion as honey either as a standalone or adjunct therapy in the treatment of osteoarthritis. The short duration of this study did not allow for obvious histo-pathological changes. Further studies should be carried out on a longer duration to allow for histo-pathological changes to occur and then assess the effect of honey on them.

Statement of authorship

HO: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Project administration; Resources; Visualization; Writing - original draft; Writing - review & editing.

BV: Methodology; Supervision; Validation; Writing - review & editing.

HO and BV contributed to the conception and design of the study, or acquisition of data, or analysis and interpretation of data, drafting the article or revising it critically for important intellectual content, and final approval of the version to be submitted.

HO and BV both agree to be personally accountable for their contributions to the study. This manuscript has been read and approved by all authors. The requirements for authorship as stated on the journal website has been met and each author believes that the manuscript represents honest work.

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Data statement

The datasets used and/or analyzed during this study are included in the published article.

Conflict of interest statement

The authors of this paper declare that they have no competing interests.

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