The Possible Osteoarthritis Disease Modifying Effect Of Trimetazidine In Rats

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osteoarthritis, inflammatory mediators, autophagy, apoptosis, and Trimetazidine.

ABSTRACT
Background: Osteoarthritis (OA) which is the most prevalent chronic joint disease and a primary contributor to pain and physical disability, impacting millions of individuals globally. It is primarily characterized by inflammation of the synovial membrane, degradation of articular cartilage, formation of osteophytes, and. remodeling of subchondral bone.
Aim: To evaluate OA disease modifying effect of trimetazidine administration on an experimental model of osteoarthritis. By assessment of histopathological changes, effect on autophagy, effect on apoptosis and the potential trimetazidine anti-inflammatory effect in rat model of osteoarthritis.
Design: The surgical induction of OA was performed on rats. The medial collateral ligament was exposed and transected through a medial midline skin incision, and the medial meniscus is reflected medially toward the femur and then cut.
The rats were divided into 3 groups as follow: group 1: control normal rats, group 2: osteoarthritic non treated rats and group 3: Trimetazidine treated osteoarthritic rats.
Results: Compared to the osteoarthritic non treated group, trimetazidine treated OA group showed a significant limitation of elevated histological score and histopathological changes, limitation of decreased LC3 expression, limitation of increased Caspase 3 expression and limitation of increased MMP-13, TNF-α and No.
Conclusion: Trimetazidine could be a promising DMOAD. Accordingly, trimetazidine may be a good choice particularly for elderly patients who often have coexisting cardiovascular conditions alongside OA. Possibly through enhancement of autophagic activity, inhibition of apoptosis, anti-inflammatory, anti-oxidative and anti-catabolic effect.

Introduction

Osteoarthritis (OA) which is the most prevalent chronic joint disease and a primary contributor to pain and physical disability, impacting millions of individuals globally. It is primarily characterized by inflammation of the synovial membrane, degradation of articular cartilage, formation of osteophytes, and. remodeling of subchondral bone (Allen, Thoma and Golightly, 2022).

Osteoarthritis involves an imbalance between pro-inflammatory and anti-inflammatory processes, leading to inflammation and cartilage degradation. Key inflammatory cytokines such as IL-1β and TNF-α are critical to OA progression (Nguyen et al., 2017). MMP-13, the primary enzyme in cartilage degradation, degrades type II collagen, proteoglycans (Sun et al., 2015). Nitric oxide further exacerbates OA by promoting pro-inflammatory cytokine expression, inhibiting collagen and proteoglycan synthesis, and inducing chondrocyte apoptosis (Abramson, 2008).

Autophagy is an intracellular degradation pathway essential for maintaining cellular homeostasis by removing cytoplasmic components such as long-lived proteins, organelles, and pathogens. It is also enhanced during processes like cell differentiation and development and under certain pathological conditions, where it plays a protective role against diseases (Smith, 2018). Autophagy can restore the function of damaged

chondrocytes, thereby alleviating the onset and progression of osteoarthritis, suggesting that targeting autophagy may offer novel treatment strategies for OA (Ren and Zhang, 2018).

Apoptosis, or programmed cell death, plays a crucial role in maintaining cellular homeostasis and regulating cell populations during development. Dysregulation of apoptosis can lead to various pathological conditions. Caspases are key mediators of apoptosis, with caspase-3 serving as the primary regulator (D'Arcy, 2019). In osteoarthritis, chondrocyte apoptosis significantly contributes to disease progression due to the limited self-repair capacity of chondrocytes to maintain the extracellular matrix. Consequently, targeting chondrocyte apoptosis represents a promising therapeutic strategy for managing OA. One potential approach to inhibit apoptosis is the induction of autophagy (Lee et al., 2024).

Current osteoarthritis treatments aim only to alleviate pain associated with the disease and typically involve a combination of pharmacological and non-pharmacological treatment. Non-pharmacological methods include exercise and rehabilitation, while pharmacological treatments mainly involve the use of paracetamol, NSAIDs, and opioids (Jiang et al., 2024). Despite numerous research on the efficacy of various disease-modifying agents, there are currently no approved disease-modifying agents approved for repairing and regenerating tissues (Rodriguez-Merchan, 2023). Therefore, development of disease-modifying osteoarthritis drugs (DMOADs) offer potential therapeutic targets, aiming to facilitate the repair and regeneration of joint tissues.

Trimetazidine (TMZ) is an approved anti-ischemic agent for the treatment of angina pectoris. The protective effects of TMZ in cardiovascular diseases are primarily attributed to the inhibition of β-oxidation of free fatty acids. TMZ further sustains ATP production at less oxygen demand, hence improving cardiac tissue's metabolic state. In the present study, trimetazidine is chosen as a potential DMOAD as TMZ can enhance autophagy (Zhang et al., 2016). TMZ has anti- apoptotic effects and also show antioxidant effect as well as can decrease the levels of inflammatory markers (Gupta et al., 2021).

Materials and methods

Drugs and chemicals: Trimetazidine (Metacardia tablets 20mg) was purchased from Global NAPI pharmaceuticals, Egypt. Rat MMP 13 (ELISA) kit from Novus biologicals com., USA. Rat Interleukin 1β (ELISA) kit from Elabscieence com., USA.Rat TNF-α 13 (ELISA) kit, from bioLegend com., USA. Nitric oxide kit, Bio-diagnostic com., Egypt. Anti-LC3 and anti-caspase-3 antibody immunohistochemistery kit from Servicebio com., China.

Experimental animals

Twenty-four adult male Sprague-Dawley rats, weighing 250-300 grams each were purchased from the medical experimental research centre (MERC), Faculty of Medicine, Mansoura University and were kept in controlled temperature 24 ± 2°C, humidity 50 ± 5% and 12-h light–dark cycle, with free access to food and water ad libitum. The study design and protocol were approved by Mansoura Faculty of Medicine, Institutional Research Board (IRB) under the code no: MDP.22.05.103.

Surgical Induction of OA

The surgical induction of OA was performed on 16 rats while the remaining 8 rats were not operated and served as normal control. The animals were anesthetized intraperitonially by using ketamine (50mg/kg) and maintained with 2% isoflurane inhalation. The left knee of the rats was shaved, sterilized by betadine solution. The medial collateral ligament was exposed and transected through a medial midline skin incision, and the medial meniscus is reflected medially toward the femur and then cut (Janusz et al., 2002).

Experimental design: The rats were divided into 3 groups as follow:

Group 1: control non osteoarthritic rats taking saline by oral gavage daily for six weeks.

Group 2: control osteoarthritic rats taking saline by oral gavage daily starting from 1st day of surgery for six weeks.

Group 3: OA rats were treated with trimetazidine dissolved in saline at a dose 30 mg /kg/ day by oral gavage daily starting from 1st day of surgery for six weeks (Zhang et al., 2016).

Assessment:
At the end of the sixth week, rats were sacrificed. Development of OA was assessed by histopathological changes developed in the affected joint. We studied the expression of LC3 (autophagy marker) and caspase 3 (apoptotic marker). In addition, blood samples were taken for measurement of biochemical markers that affect the disease process and have role in the pathogeneses of osteoarthritis as pro-inflammatory cytokines (IL-1β and TNFα), proteolytic enzymes (MMP-13) and nitric oxide (NO).

Histopathological assessment
The preparation of knee joint specimens for the histopathological examination was done by the extraction of the skin then osteotomy above and under the knee joint. The fixation of the specimens was done, then decalcified with EDETA. Five-micron tissue sections were cut and stained with haematoxylin and eosin (H&E) as described by Schmitz et al. (2010) for examination of cartilage and subchondral bone. Grading of histological lesions performed according to the scoring system of Khan et al. (2013).

Immunohistochemistry for assessment of autophagy and apoptosis in articular cartilage specimens:
Knee specimens were sectioned into 5-µm cross-sections and prepared for immunohistochemical analysis. The sections were deparaffinized in xylene, hydrated through graded alcohols, washed in ethanol and washed in deionized water. Antigen unmasking was performed by heating slides in 10 mM sodium citrate buffer with 0.01% EDTA at pH 6.0, followed by cooling and washing. The sections were then immunostained using specific antibodies to analyze LC3 content and distribution, as well as caspase-3 content and distribution.

Biochemical markers assessment
Blood was withdrawn from the heart of rats by 5 ml syringe. Most blood was put in a dry test tube, and then sera were separated and collected in Eppendorf tube and kept frozen at -20°C till the time of measurement of MMP13, IL-1β, TNF-α by enzyme-linked immunosorbent assay (ELISA) kit and NO by colorimetric method.

Statistical Analysis
The results were statistically analyzed using the Statistical Package for Social Science (SPSS) program version 25. The parametric results were expressed as Mean ± SD. One-way analysis of variance (ANOVA) followed by post hoc Tukey's multiple comparisons was used for statistical analysis between groups. For all above mentioned statistical tests the results were considered significant when the probability of error is less than or equal 5% ($p \leq 0.05$).

Results

Effect of trimetazidine on histopathological assessment
The histopathological examination of the control normal rats showed intact histological picture; intact superficial layer, joint cartilage with normal architecture, rounded chondrocytes in lacunae, and average sized bone marrow spaces (figure 2A, a) and showed histopathological score (0 ± 0).

The histopathological examination of the osteoarthritic non treated rats showed loss of superficial layer, distortion of the normal architecture enlarged bone marrow spaces, cartilage replacement with fibrous tissue, and small chondrocytes. (figure2 B,b) and showed a significant increase in histopathological score (5.3 ± 1.4) as compared to that of control normal rats.

The histopathological examination of the trimetazidine treated OA group showed normal architecture with normal sized bone marrow spaces, and rounded chondrocytes in lacunae (figure2 C,c). Trimetazidine treated OA group showed a significant increase in histological score (1.1± 0.29), This increase was significantly lower than osteoarthritic non treated group but still significantly higher than the control normal group (figure 1).

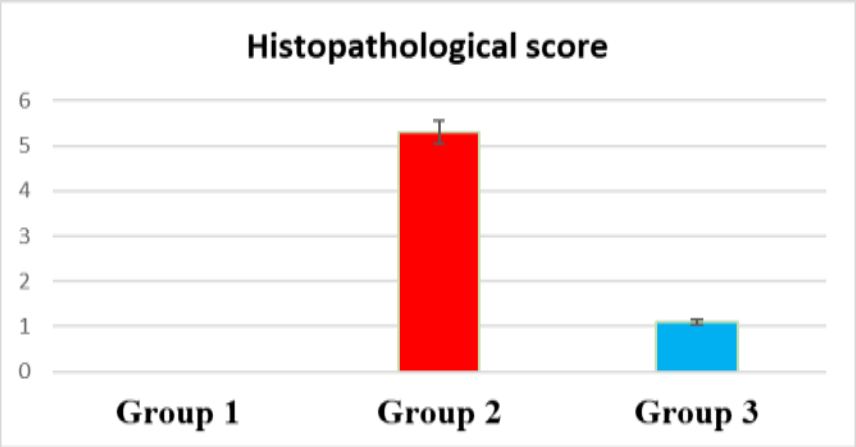

Figure-1: Histopathological score

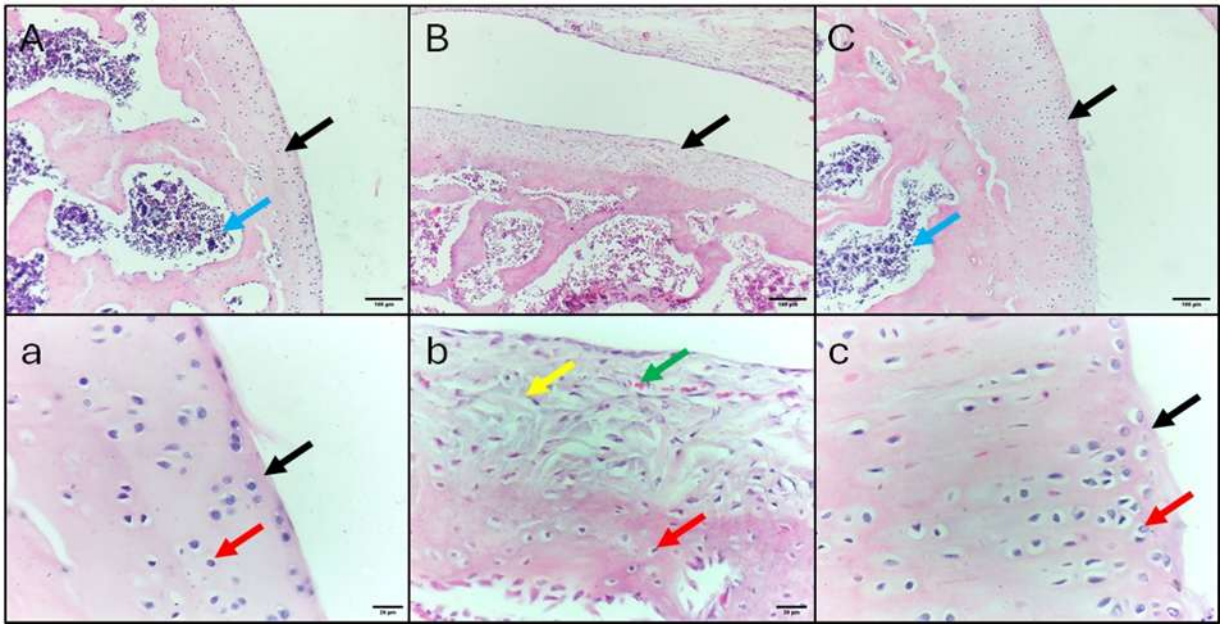

Figure-2: H&E staining micrographs of joint sections

(A, a) Normal control group, showing joint cartilage with normal architecture, rounded chondrocytes in lacunae, and average sized bone marrow spaces.

(B, b) Osteoarthritis non treated group, showing distortion of the normal architecture enlarged bone marrow spaces, cartilage replacement with fibrous tissue, small chondrocytes and hemorrhage.

(C, c) Trimetazidine treated group, showing normal architecture with normal sized bone marrow spaces, and rounded chondrocytes in lacunae.

Black arrow: joint cartilage, Blue arrow: bone marrow spaces, Red arrow: chondrocytes, Yellow arrow: fibrosis, Green arrow: hemorrhage.

Upper raw original magnification 10X, lower raw 40X and scale bar 100 µm and 20 µm respectively.

Assessment of Immunohistochemistry

Effects of trimetazidine on light chain 3 (LC3) expression by immunohistochemistry in osteoarthritic rats: Control normal group showed level of LC3 expression (18.3 ± 3.3). Surgical induction of OA in rats produced a statistically significant decrease in the level of LC3 expression (0.59 ± 0.21) as compared to the control normal

rats. Trimetazidine treated OA group showed a statistically significant increase of the LC3 expression (9.7± 2.1). This increase was significantly higher than osteoarthritic non treated group but still significantly lower than the control normal group (figure3,5).

Effects of trimetazidine on Caspase-3 expression by immunohistochemistry in osteoarthritic rats:
Control normal rats showed level of Caspase-3 expression (5.3 ± 1.6). Surgical induction of OA in rats produced a statistically significant increase in the level of Caspase-3 expression (30.4±2.9) as compared to the control normal rats. Trimetazidine treated OA group showed a statistically significant increased Caspase-3 expression (10.2± 1.8). This increase was significantly lower than osteoarthritic non treated group but still significantly higher than the control normal group, (figure 4,6).

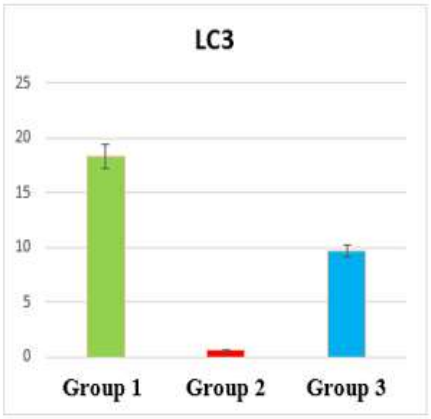

Figure-3: LC3 expression

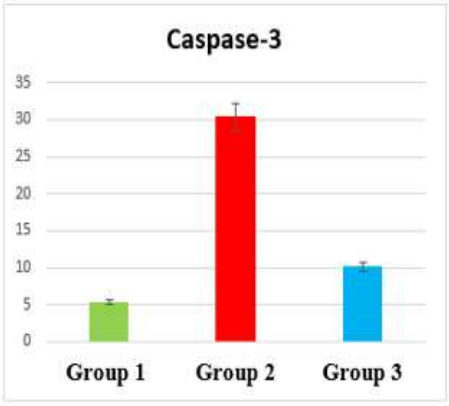

Figure-4: Caspase-3 expression

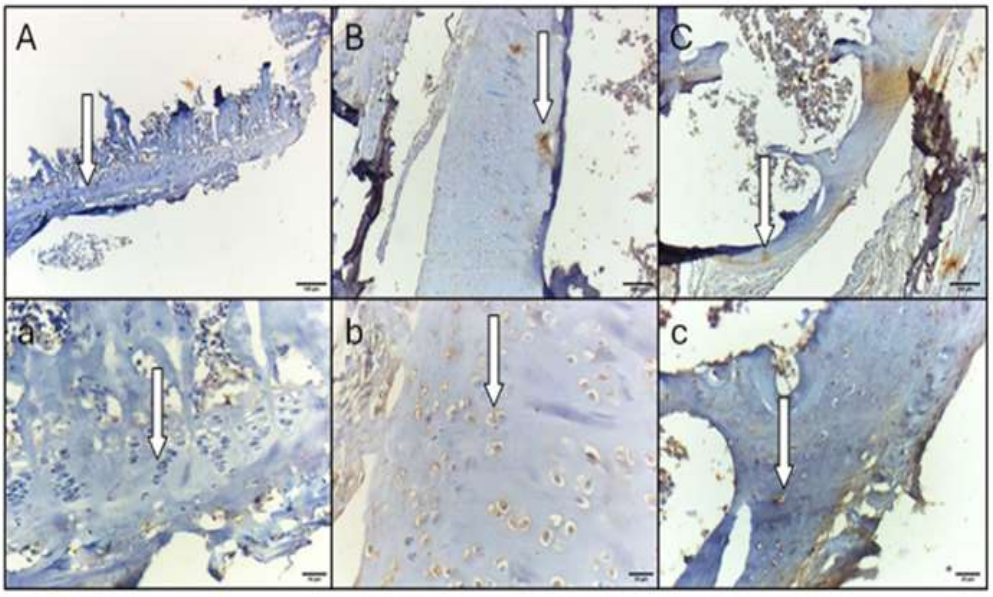

Figure-5: LC3 immunohistochemical staining micrographs of joint sections

(A, a) Normal control group, showing joint cartilage positive immunoreactivity in the chondrocytes.
(B, b) Osteoarthritis non treated group, showing no immunoreactivity in all chondrocytes.

(C, c) Trimetazidine treated group, showing focal positive immunoreactivity in some of the chondrocytes.

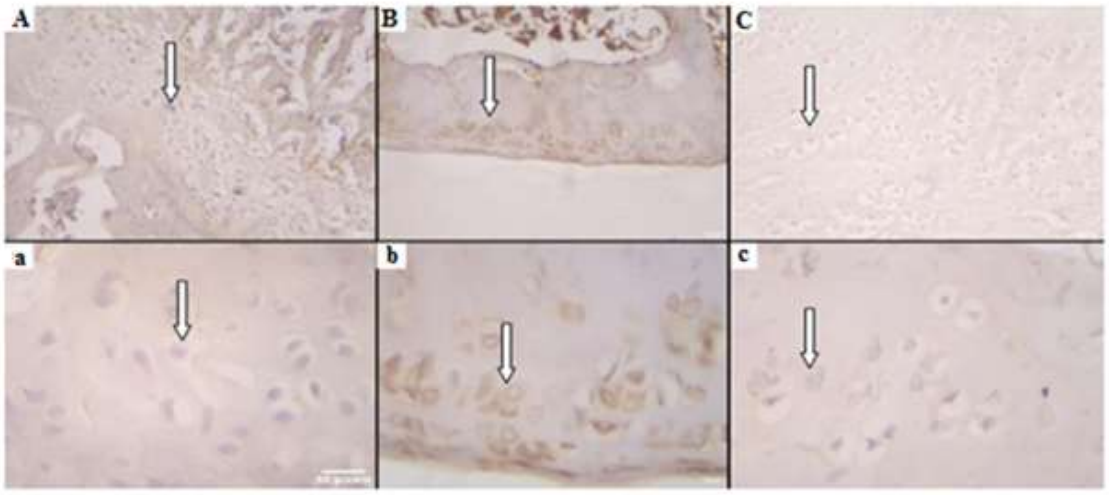

Figure-6: Caspase immunohistochemical staining micrographs sections of

A, B, C: low power of LC3 expression *100
a, b, c: high power of LC3 expression *400.

(A, a) Normal control group, showing joint cartilage no immunoreactivity in the chondrocytes.
(B, b) Osteoarthritis non treated group, showing positive immunoreactivity in all chondrocytes (brown color).
(C, c) Trimetazidine treated group, showing mild positive immunoreactivity in some of the chondrocytes.
a, b, c: low power of caspase expression *100
A, B, C: high power of caspase expression *400

Biochemical markers assessment

A) Measurement of interleukin1- β
Control normal rats showed serum IL1-β (25.8 ± 6. 2 ng/ml). Surgical induction of OA in rats produced a significant rise in serum IL1-β (102.2 ± 19.3 ng/ml) as compared to the control normal rats. Trimetazidine treated OA group showed statistically significant increase in serum IL1-β (42.5 ± 11.3 ng/ml). This increase was significantly lower than osteoarthritic non treated group but still significantly higher than the control normal group, (Figure 7).

B) Measurement of matrix metalloproteinase 13:
Control normal rats showed serum level of MMP-13 (0.97 ± 0.23 pg/ml). Surgical induction of OA in rats produced a statistically significant increase in serum level of MMP13 (2.6 ±0.46 pg/ml) as compared to the control normal rats.Trimetazidine treated OA group showed increased in serum level of MMP13 (1.3± 0.29 pg/ml). This increase was significantly lower than osteoarthritic non treated group, (figure8).

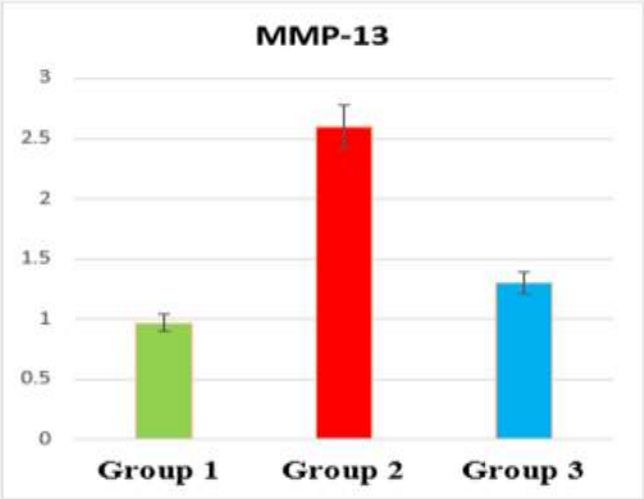

Figure-8: Matrix metalloproteinases 13

C) Measurement of tumor necrosis factor- alpha

Control normal rats showed serum TNF-α level (21.6 ± 4.6 pg/ml). Surgical induction of OA in rats produced a statistically significant increase in serum TNF-α (82.8 ± 21.3 pg/ml) as compared to the control normal rats. Trimetazidine treated OA group showed a statistically significant increased serum TNF-α level (44.4± 11.2 pg/ml). This increase was significantly lower than osteoarthritic non treated group, but still significantly higher than the control normal group, figure 9).

D) Measurement of nitric oxide

Control normal rats showed serum No (0.85 ± 0.14 pg/ml). Surgical induction of OA in rats produced a statistically significant increase in serum No (2.0 ±0.34 pg/ml) as compared to the control normal rats, (Table11& figure15). Trimetazidine treated OA group showed a statistically significant increased serum No (1.3± 0.27 pg/ml). This increase was significantly lower than osteoarthritic non treated group, but still significantly higher than the control normal group, (figure10).

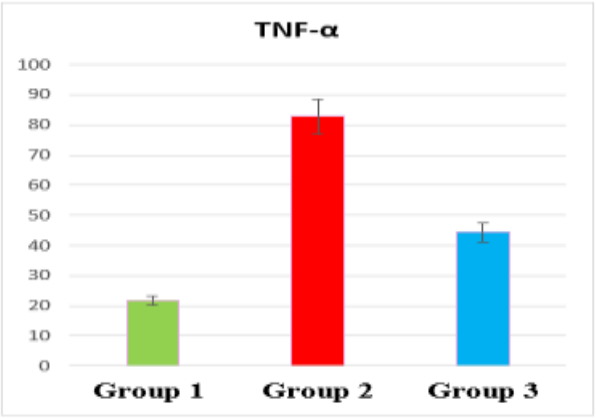

Figure-9: Tumor necrosis factor- alpha

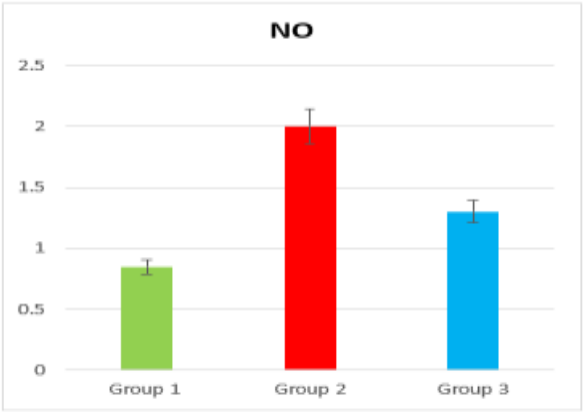

Figure-10: Nitric oxide

Discussion

Osteoarthritis is the most prevalent chronic degenerative joint disease, which is a primary contributor to ongoing pain and prolonged disability in adults. Current OA treatments focus on pain relief using pharmacological

treatment (e.g., paracetamol, NSAIDs, opioids) and non-pharmacological approaches (Derry et al., 2016). Despite numerous studies on the efficacy of various disease-modifying agents, no approved therapies are currently available to repair or regenerate joint tissues (Rodriguez-Merchan, 2023).So, development of disease-modifying osteoarthritis drugs (DMOADs) offer potential therapeutic targets, aiming to facilitate the repair and regeneration of joint tissues.

In the present study, trimetazidine is chosen as a potential DMOAD as TMZ can enhance autophagy (Zhang et al., 2016). TMZ has anti- apoptotic effects, anticatabolic and also show antioxidant effect as well as can decrease the levels of inflammatory markers (Gupta et al., 2021).

The destabilization of the medial meniscus (DMM) model has emerged as a standard for studying the initiation and progression of post-traumatic osteoarthritis (Culley et al., 2021). Therefore, in the present study, osteoarthritis was surgically induced in rats' knees through medial collateral ligament transection and meniscal tear procedures, as described by Janusz et al. (2002).

In the present study histopathological examination and scoring was done to all groups proved that control normal group showed histological picture which is completely normal; intact superficial layer, joint cartilage with normal architecture, rounded chondrocytes in lacunae, and average sized bone marrow spaces.

Experimental osteoarthritis was successfully induced in rats through surgical transection of the medial collateral ligament and medial meniscal tear. In the untreated OA group, histopathological analysis revealed characteristic OA changes, including loss of the superficial cartilage layer, disrupted architecture, enlarged bone marrow spaces, fibrous tissue replacement, and small chondrocytes, confirming OA development through a significantly elevated histopathological score compared to controls. Our findings align with those of Takahashi et al. (2024) who reported chondrocyte apoptosis, matrix loss, thinning of the superficial cartilage layer, and osteocyte death in subchondral bone as hallmarks of OA.

In trimetazidine treated OA group, the histopathological examination showed normal architecture with normal sized bone marrow spaces, and rounded chondrocytes in lacunae. Trimetazidine treated OA group showed a significant limitation of elevated histological score and histopathological changes that occur in osteoarthritic non treated group. Our results are in agree with Metwally and Nawar (2022) who reported that the trimetazidine-treated group displayed normal structured chondrocytes and normal bone trabeculae. This outcome can be attributed to the anti-inflammatory and antioxidant properties of trimetazidine.

Autophagy, an intracellular degradation mechanism essential for maintaining energy metabolism homeostasis within cells, has been shown to restore the function of damaged chondrocytes. This process helps n preventing and slowing the progression of osteoarthritis and offers novel approaches for OA treatment by targeting autophagy (Ren and Zhang, 2018). In mammalian systems, the primary biological marker for detecting autophagy is the microtubule-associated protein light chain 3 (LC3), which makes up the autophagosomal membrane (Duan, Xie and Liu, 2020).

In the present study, the induction of OA in osteoarthritic non treated group was associated with decrease in LC3 expression as compared to control normal group These effects came in agreement with Feng et al., (2020) who reported that as OA progresses, autophagy decreases. The upregulation of mTOR, a principal inhibitor of autophagy, suppresses autophagy signal transduction in articular cartilage. This suppression diminishes autophagy's protective role, thereby accelerating cartilage degeneration (Vasheghani et al., 2015).

On the other hand, in the present study, trimetazidine produced significant increase of LC3 expression in trimetazidine treated group compared to osteoarthritic non treated group. These effects came in agreement with Ferraro et al., (2013) who reported that trimetazidine induces autophagy. It was found that stimulation of autophagy in chondrocytes through intra-articular administration of resveratrol, an agent that induces autophagy, can markedly slow down the degeneration of articular cartilage in a DMM OA mouse model. Therefore, autophagy serves as a protective mechanism for chondrocytes against degradation (Qin et al., 2017).

Chondrocyte apoptosis is considered a critical factor in the progression of OA, as these cells have limited self-repair capacity to preserve the extracellular matrix. Consequently, targeting chondrocyte apoptosis is a promising therapeutic strategy for OA treatment. Several mediators and targets, including the induction of autophagy, have been identified to inhibit apoptosis (Lee et al., 2024).

In the present study, the induction of OA in osteoarthritic non treated group was associated with increase

in Caspase 3 expression as compared to control normal group which indicate activation of apoptosis. These effects came in agreement Zhang et al., (2021) who reported that chondrocyte apoptosis is a hallmark of OA and there is a strong relationship exists between them. Caspase-3 plays a key role in the regulation of apoptosis, and research has shown that its expression is significantly increased in OA cartilage (Almeida et al., 2016).

On the other hand, in the present study, trimetazidine treated OA group produced significant limitation of increased Caspase 3 expression demonstrated in osteoarthritic non treated group which indicate suppression of apoptosis. These effects came in agreement with Amberg et al., (2017) who revealed that trimetazidine has anti-apoptotic properties.

In this study measurement of IL-1 β in rat serum was done in all groups. Following induction of OA in rats, it was found that there is significant increase of IL-1 β in osteoarthritic non treated group compared to control normal group. This increase in level of IL1- β supported with other previous studies as described by Jia et al. (2022) who reported that increased IL-1β, a key proinflammatory cytokine in OA pathogenesis, is commonly observed in OA.

Trimetazidine treated OA group produced significant limitation of increased IL-1 β demonstrated in osteoarthritic non treated group. Our results are in agree with (Tanoglu et al., 2015; Hohensinner et al., 2021) who reported that trimetazidine can inhibit the release of cytokines such as IL-1β.

In the present study measurement of MMP13 in rat serum was done in all groups. After induction of OA in rat, it was found that there was significant increase of MMP-13 in osteoarthritic non treated group compared to control normal group. These findings are consistent with Li et al. (2017) who reported that in an experimental OA model MMP-13 levels are closely associated with pathological changes characteristic of OA progression. Overexpression of MMP-13 can trigger OA onset by causing excessive degradation of the extracellular matrix.

Trimetazidine treated OA group produced significant limitation of increased MMP-13 showed in osteoarthritic non treated group. These results are also in line with Gong et al., (2018) who reported that trimetazidine can inhibit oxidative stress, which is associated with the downregulation of metalloproteinase expression. MMP-13 is a key enzyme responsible for cartilage degradation, can degrade all of the extracellular matrix's constituents including collagen and proteoglycans (Zeng et al., 2015).

In the present study, it has been found that there was significant increase of TNF- α in osteoarthritic non treated group compared to control normal group. The increase in level of TNF-α in OA came in agreement with Li et al., (2018) who demonstrated that TNFα is associated with the progression of osteoarthritis. TNF-α is a potent proinflammatory cytokine that plays a crucial role in OA-related inflammatory responses by promoting the synthesis of proteolytic enzymes and inhibiting the production of articular tissue matrix macromolecules.

Trimetazidine treated OA group produced significant limitation of increased TNF-α presented in osteoarthritic non treated group. These results are also in line with Su et al., (2017) who reported that TMZ suppressed the elevation of inflammatory markers, including TNF-α.

In the present study, the induction of OA was associated with significant increase of NO in osteoarthritic non treated group as compared to control normal group. These effects came in agreement with Abramson, (2008) who reported that osteoarthritis is characterized by elevated levels of oxidative stress markers, which inhibit the synthesis of collagen and proteoglycans, activate matrix metalloproteinases, increase cartilage susceptibility to further oxidative damage, and induce apoptosis.

Trimetazidine treated OA group produced significant limitation of increased NO showed in osteoarthritic non treated group. These results are also in line with Abdel-Salam et al., (2011) who reported that trimetazidine administration significantly reduced NO levels following lipopolysaccharide-induced oxidative stress in mice.

Conclusion

To summarize, our study demonstrates that trimetazidine could maintain the normal architecture. It increases the LC3 expression so induces autophagy and decreases Caspase-3 expression indicate inhibition of apoptosis. Trimetazidine exerts a significant anti-catabolic, anti-inflammatory and anti-oxidative effect by reducing MMP 13, IL-1β TNF-α and No. So, Trimetazidine can protect chondrocyte. These findings support the rationale for investigating trimetazidine as a potential DMOAD. Based on our findings, trimetazidine could be a promising

DMOAD and may be a good choice particularly for elderly patients who often have coexisting cardiovascular conditions alongside OA.

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