IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

# EFFECT OF METFORMIN USAGE IN EXPERIMENTAL RAT ANIMALS INDUCED WITH MEDIAL MENISCUS DESTABILIZATION: A SYSTEMATIC REVIEW

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***Abstract:*** Osteoarthritis (OA) is a degenerative disease that occurs due to the breakdown of joint cartilage in weight-bearing joints. In various in vivo and in vitro studies metformin showed benefits of delaying aging and prolonging life and has great potential for the treatment of other disorders. Because the way metformin works activates AMP-Activated Protein Kinase (AMPK) which has been assumed to be a potential target therapy for Osteoarthritis, it is possible that Metformin can be a therapy for Osteoarthritis. There have been several studies on experimental animal models of autoimmune arthritis that show that metformin also has anti-arthritic activity and inhibits bone degradation. To analyze the effect of metformin use in experimental rat animals that have been induced medial meniscus destabilization. Literature searches were obtained from PubMed and Google Scholar databases. The population of this study was experimental rats that had been induced medial meniscus destabilization. After reviewing relevant articles, 5 articles were determined to be eligible and were high quality articles. Researchers analyzed the effect of metformin administration on rat animals that have been induced medial meniscus destabilization. Metformin significantly affects the progression of Osteoarthritis in mice. Further studies in humans using the same evaluation parameters need to be conducted to prove the effect of metformin administration on Osteoarthritis progressivity in humans.

***Index Terms*** **- Osteoarthritis, Metformin, Mice, DM.**

**Introduction**

Osteoarthritis (OA) is a degenerative joint disease that occurs due to the destruction of joint cartilage [1] and is long-term chronic. Osteoarthritis is characterized by reduced articular cartilage in focal areas within the joint synovial, associated with bone hypertrophy (osteocytes and subchondral bone sclerosis) and thickening of the capsule. Osteoarthritis usually affects weight-bearing joints, causing the bones to rub together and leading to stiffness, pain and movement disorders.

Pain and irreversible cartilage damage cause health problems such as psychological changes, limited social interaction, decreased physiological abilities, decreased work productivity and can reduce the quality of life. According to research, Osteoarthritis is the number 5 disease that causes Years of Life Disability in women and developed countries, and number 8 in developing countries[2] According to research, an estimated 8.5 million people in the UK suffer from Osteoarthritis disease, causing pain and even disability [3], so the treatment of Osteoarthritis has an important role to eliminate arthralgia, prevent disease progression, and irreversible cartilage degradation and aggravate pain (Feng et al., 2020).

Metformin is known to have been used as an antidiabetic drug. In various in vivo and in vitro studies, it has been shown that metformin can delay aging and prolong life and has great potential for the treatment of other disorders. However, the role of metformin in Osteoarthritis is unknown. Because the mode of action of metformin activates AMP-Activated Protein Kinase (AMPK) which has been assumed to be a potential target therapy for Osteoarthritis, it is possible that Metformin can be a therapy for Osteoarthritis [5], [6] There have been several studies on experimental animal models of autoimmune arthritis that show that metformin also has anti-arthritic activity and inhibits bone degradation [4], [5]. However, the results of other studies showed no significant difference between metformin prescribing and the diagnosis of Osteoarthritis [7].

Based on the description above, the authors are interested in conducting a systematic study related to the progressivity of osteoarthritis after the use of metformin and also the effectiveness of metformin in experimental rat animals that have been induced medial meniscus destabilization.

## I. METHODS

This study used a retrospective observational research design with a *systematic review* and/or *meta-analysis* approach, where all variable data were collected from previously conducted studies. Data synthesis and/or data analysis of the collected studies were conducted. This study data was collected from previous research data in the form of clinical trial research journals collected from PubMed and Google Scholar *databases*.

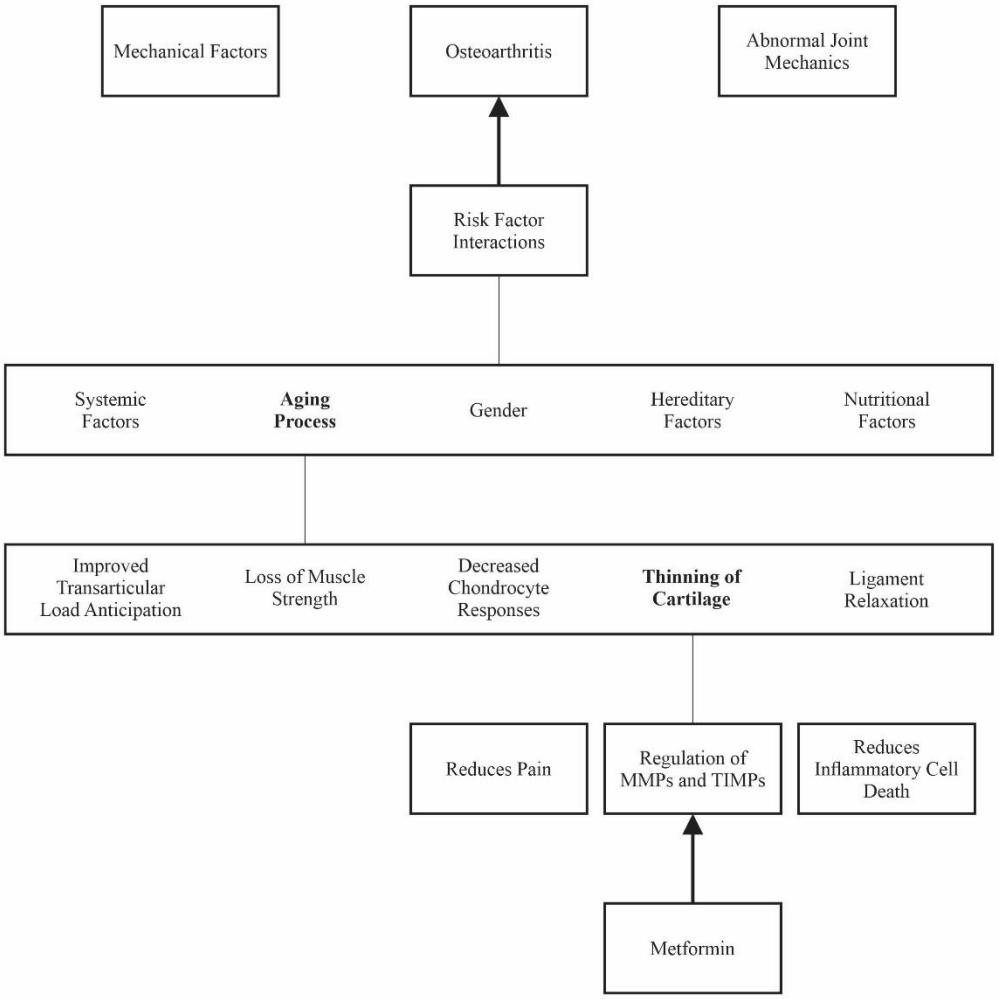


Figure 1. Conceptual Framework of Systematic Review

## II. RESULT AND DISCUSSION

From both databases, 38 studies were found with search terms in the title, abstract, and keywords as follows: "Metformin" AND "Mice" OR "Mouse" OR "Rat" AND "Osteoarthritis". In the literature search, search limits were used:

1. Year of article publication between 2017-2022 (Google Scholar and PubMed)
2. Research article (Google Scholar)
3. Full text, Clinical Trial (PubMed)

From 38 studies, 10 studies were eliminated. The studies were then screened through the title and abstract, and studies were found that did not meet the PICO and sample criteria, which were caused by, among others:

1. The study was not conducted on rats that had been induced to destabilize the medial meniscus.
2. The research output is not the effect of metformin on osteoarthritis progressivity.
3. Research in languages other than English or Indonesian
4. Research published within the last five years or more
5. Research with a non-experimental study design

After the screening process, 8 studies were obtained that were suitable and potential to be reviewed in this review. after reviewing the full article, some of them were excluded because

1. Research conducted in abstract form only
2. The research is a study protocol

The final selection of 5 studies that met the criteria was included in this systematic review. The diagram for this process can be seen in Figure 1 which shows the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) used for the selection of studies that can be used for this study. These five studies scored low risk after assessing study quality using the ROBINS-I instrument.

### 3.1 Study Sample Characteristics

Table 1. Reviewed Studies

| No. | Title | Author | Year | Population | Intervention | Control |
|---|---|---|---|---|---|---|
| 1 | Metformin attenuates cartilage degeneration in an experimental osteoarthritis model by regulating AMPK/mTOR | Feng *et al* | 2019 | 8-week-old male C57BL/6 mice (n: 72) with medial meniscus destabilization (DMM) method on the right knee | - Rats with dose-dependent intervention of metformin 100 mg/kgBB/day<br>- Rats with dose-dependent intervention of metformin 200 mg/kgBB/day | Mice that have gone through the DMM process without metformin intervention |
| 2 | METFORMIN LIMITS OSTEOARTHRITIS DEVELOPMENT AND PROGRESSION THROUGH ACTIVATION OF AMPK SIGNALING | Li *et al* | 2020 | 10-week-old wild-type mice with destabilized medial meniscus (DMM) On the right knee | Mice were given activated protein kinase (AMPK) and metformin 4 mg per day in drinking water for 12 weeks. | Wild-type mice not administered AMPK and metformin |
| 3 | EXPLORATION OF METFORMIN AS A NOVEL THERAPY FOR OSTEOARTHRITIS : PREVENTING CARTILAGE DEGENERATION AND REDUCING PAIN BEHAVIOR | Li *et al* | 2020 | 10-week-old male C57BL/6 mice (n: 80) with medial meniscus destabilization (DMM) of the right knee | Rats were given intragastric and intraarticular metformin and DMM was performed at the following doses:<br>- Intragastric Metformin (200 mg/kg) 3 days after DMM given daily for 8 weeks<br>- Intraarticular metformin (0.01 mmol/kg) was injected 3 days after DMM twice a week for 8 weeks. | Rats were administered intragastric and intraarticular saline at the following doses:<br>- Intragastric Saline (10 ml/kg) 3 days after DMM given daily for 8 weeks<br>- Intraarticular metformin (1 mmol/kg) was injected 3 days after DMM twice a week for 8 weeks. |
| 4 | Metformin Mitigates Cartilage Degradation by Activating AMPK/SIRT1-Mediated Autophagy in a Mouse Osteoarthritis Model | Wang *et al* | 2020 | 8-week-old male C57BL/6 mice (n:56) with medial meniscus destabilization (DMM) method on the right knee | C57BL/6 male mice with destabilization method (DMM) and medial tibial menisco ligament in the right knee transected<br>(1) not given metformin and<br>(2) were given metformin intra-articularly at a dose of 1.65 g/ml given every 3 days for 8 weeks | C57BL/6 male mice without DMM method and medial menisco tibial ligament of the right knee not transected |

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|---|---|---|---|---|---|---|
| **5** | Metformin Attenuates Monosodium-Iodoacetate-Induced Osteoarthritis via Regulation of Pain Mediators and the Autophagy-Lysosomal Pathway | Na *et al* | 2021 | 7-week-old male rats (n:6) monosodium iodoacetate on the right knee | Six rats induced osteoarthritis with monosodium iodoacetate were given metformin at a dose of 100 mg/kg orally. | Rats induced osteoarthritis with monosodium iodoacetate not given metformin |

Table 2. Results of Reviewed Studies

| **No.** | **Title** | **Author** | **Year** | **Results** |
|---|---|---|---|---|
| **1** | Metformin attenuates cartilage degeneration in an experimental osteoarthritis model by regulating AMPK/mTOR | Feng *et al* | 2019 | DMM-induced joint cartilage degeneration was significantly reduced in rats given dose-dependent metformin intervention. Confirmed by lower OARSI scores in metformin-treated rats.<br>Week 2 OARSI score<br>1. Control Mice >2<br>2. Rats treated with metformin 100 mg/kgBB/day <2<br>3. Mice treated with metformin 200 mg/kgBB/day <1<br>Week 5 OARSI score<br>1. Control Mouse 3<br>2. Mice treated with metformin 100 mg/kgBB/day <3<br>3. Rats treated with metformin 200 mg/kgBB/day <2<br>Week 10 OARSI score<br>1. Control Rat 6<br>2. Rats treated with metformin 100 mg/kgBB/day < 4<br>3. Rats treated with metformin 200 mg/kgBB/day <3 |
| **2** | Metformin limits osteoarthritis development and progression through activation of AMPK signalling | Li *et al* | 2020 | Metformin at 4 mg per day in drinking water for 12 weeks. Based on the OARSI score, cartilage damage was mild at 6 weeks and became severe at 12 weeks after the DMM procedure.<br>Week six OARSI score<br>1.Mice without metformin administration<br>- Without DMM < 2<br>- DMM < 4<br>2.Mice were given metformin since 2 weeks before DMM<br>- Without DMM < 2<br>- DMM = 2<br>3.Mice were given metformin since 2 weeks after DMM<br>- Without DMM < 2<br>- DMM < 3<br><br>Week 12 OARSI score<br>4.Mice without metformin administration<br>- Without DMM < 2<br>- DMM < 4<br>5.Mice were given metformin since 2 weeks before DMM<br>- Without DMM < 2<br>- DMM < 4<br>6.Mice were given metformin since 2 weeks after DMM<br>- Without DMM < 2<br>- DMM=4 |
| **3** | Exploration of metformin as a novel therapy for osteoarthritis: preventing cartilage degeneration and | Li *et al* | 2020 | Samples from the IGS (*Intragastric Saline Administration*) and IAS (*Intraarticular Saline injection*) groups showed more severe articular cartilage damage compared to samples from the IGM (*Intragastric Metformin Administration*) or IAM (*Intraarticular Metformin Injection)* groups.<br>Week 8 OARSI score<br>Rats with IGS (10 ml/kg) <6 |

| | | | | |
|---|---|---|---|---|
| | reducing pain behavior | | | Rats with IGM (200 mg/kg) <4<br>Rats with IAS (1 ml/kg) <6<br>Mice with IAM (0.1 mmol/kg) <4 |
| **4** | Metformin Mitigates Cartilage Degradation by Activating AMPK/SIRT1-Mediated Autophagy in a Mouse Osteoarthritis Model | Wang *et al* | 2020 | There were no changes in the structure of the ACL or meniscus in rats that were not induced with DMM. Safranin-O staining and OARSI scores showed the DMM procedure caused joint cartilage damage and increased synovial membrane thickness. Intra-articular administration of metformin at a dose of 1.65 g/ml given every 3 days for 8 weeks effectively reduced joint cartilage damage compared to the group of rats not given metformin.<br>- Week eight OARSI score:<br>1.Rats without DMM = 0<br>2.DMM rats = 10<br>3.DMM rats with Metformin administration = 5 |
| **5** | Metformin Attenuates Monosodium-Iodoacetate-Induced Osteoarthritis via Regulation of Pain Mediators and the Autophagy-Lysosomal Pathway | Na *et al* | 2021 | Metformin administration reduced the OARSI score compared to the control group<br>Day 14<br>Control mice <4<br>Rats with oral metformin administration (100 mg/kg) <2 |

**3.2 Results of Descriptive Statics of Study Variables**

Based on the study of Feng et al, in 3 groups of 8-week-old male C57BL/6 mice induced osteoarthritis by the DMM method with a study duration of 10 weeks showed a reduction in the progressivity of the OARSI score in the group of mice dosed with metformin 100 mg / kgBB / day and 200 mg / kgBB / day than the control group of mice. Metformin works by activating AMPK and suppressing mTORC1 to suppress cartilage degradation and aging and suppress the development of osteoarthritis. Under physiological and pathological conditions, the mammalian Target of rapamycin (mTOR) signaling pathway plays an important role in protein translation, synthesis, cell metabolism, and stress response. When the AMPK signaling pathway is activated, AMPK will activate autophagy, along with AMPK phosphorylation blocking the phosphorylation of the mTOR signaling pathway and inhibiting its corresponding biological functions, ultimately regulating the progressivity of osteoarthritis. Thus, metformin as an activator of AMPK signaling pathway may induce polarization of AMPK signaling pathway and activation of autophagy. Immunofluorescence staining of Osteoarthritis model from Feng et al study showed, with the increase of metformin concentration, there was an increase in the expression of p-AMPK positive cells, and p-AMPK protein, and there was also a decrease in the expression of p-S6 positive cells and p-S6 protein in chondrocytes, indicating that metformin treatment dose-dependently activated AMPK signaling pathway and inhibited mTORC1 signaling pathway.

The results of the Feng et al study showed that the degradation rate of the cartilage matrix during osteoarthritis progressed significantly improved compared to the control group with increasing time and metformin concentration, and also found a decrease in the level of Metalloproteinases (MMPs) enzymes MMP-13 & MMP-3 which play a major role in degrading the cartilage matrix as the metformin concentration increases. This also occurred in chondrocyte cultures which showed the expression and mRNA levels of MMP-13 & MMP3 decreased with the addition of time and increased metformin concentration. At the same time, the expression level of cartilage matrix synthesis protein, COLII, and mRNA expression of SOX9 increased. These results suggest that metformin reduces cartilage matrix degradation and stimulates cartilage matrix synthesis.

The study of Li et al presents several study results including Intragastric and intraarticular metformin modulates pain-related in DMM-induced Osteoarthritis model, Intragastric and intraarticular metformin attenuates articular cartilage degradation in DMM-induced Osteoarthritis model, Metformin is protected from catabolism by interleukin-1β in chondrocytes and cartilage explants, AMPK activation is involved in the protective effect of metformin against catabolism by IL-1β in chondrocytes.

To examine the ultrastructure of the cartilage surface at 8 weeks after surgery, SEM evaluation of the "plateau" tibia of surgically induced Osteoarthritis rats was performed. The cartilage surfaces in rats in the non-operated and sham-operated groups were smooth with no ultrastructural changes. Rats in the IGS and IAS groups had extensive peeling of the cartilage area, exposed subchondral bone with small cracks and little Safranin O staining. Slightly exfoliated cartilage, superficial avulsion and loss of Safranin O staining were demonstrated in the IGM and IAM groups. The mean subjective scores recommended by OARSI among the IGS and IAS groups were higher compared to the IGM or IAM groups; however, the OARSI scores in the IGM or IAM groups were lower compared to the IGS or IAS groups.

Further examination was conducted to determine whether metformin could protect the catabolism of chondrocytes treated with IL-1β and cartilage explants in vitro. After treatment in a dose-response manner at 24 hours, the mRNA level of matrix MMP-13 in chondrocytes was decreased. In contrast, metformin did not significantly modulate the mRNA level of anabolic gene type II collagen alpha 1 chain (col2a1), 10 mM and 20 mM metformin significantly increased the expression level of type II collagen. Meanwhile, there was no significant change in cell viability in chondrocytes treated with 1 mM or 10 mM metformin.

10 mM metformin was chosen to treat chondrocytes as it protects against catabolism without compromising cell viability. There was no significant change in the expression level of AMPKα1 in chondrocyte cultures in the presence of IL-1β 24 h after treatment with metformin; however, the expression level of pAMPKα protein, indicated AMPK activation.

In the study of Wang et al, metformin relieves Osteoarthritis by triggering autophagy and inhibiting apoptosis in a rat DMM model. The protective effect that metformin provides is through activating the AMPKa2/SIRT1 pathway. In this study, DMM surgery increased the expression of MMP 13 and decreased the expression of type II collagen and proteoglycans, suggesting that the surgery successfully induced Osteoarthriti changes. IL-1β increased the expression of metalloproteinases and induced apoptosis in cultured chondrocytes, supporting the record that IL-1β is an important proinflammatory cytokine in the process of Osteoarthritis. In this study, decreased autophagy was observed in the knees of DMM rats, confirming the decrease of autophagy in the development of Osteoarthritis. AMPK/SIRT1 axis is the initial signaling for autophagy. Activation of AMPKa protein, shown as phosphorylation at Thr 172 residue, reduced IL-1β-induced catabolic gene expression in chondrocytes. Inhibition of AMPK/SIRT1/autophagy by either pharmacological agents or genetic approaches augments the Osteoarthritis phenotype. Activation of AMPK/SIRT1 signaling is critical in maintaining cartilage homeostasis. This study shows that metformin exerts a protective effect via SIRT3-activated PINK1/Parkin-mediated mitophagy in cultured chondrocytes. Metformin treatment reduced cell apoptosis in cultured chondrocytes and Osteoarthritis-interested mice, suggesting that metformin exerts a protective effect against cell apoptosis. In this study, inhibition of autophagy by either pharmacological or genetic approaches, reversed the protective effect of metformin. Autophagy inhibition potentiates the occurrence of apoptosis, confirming the interaction between apoptosis and autophagy.

In the Li et al study, several key observations were made in the presented study. Metformin given immediately after joint injury, limited the development of Osteoarthritis and delayed the progression of Osteoarthritis. Metformin also reduced Osteoarthritis-related pain sensitivity. Metformin upregulated AMPKα1 expression and metformin lost its chondroprotective effect in AMPKα1 KO mice; this suggests that the chondroprotective effect of metformin is mediated by activation of AMPK signaling. In addition, the progression rate of Osteoarthritis during the period of 6 to 12 weeks after DMM surgery was analyzed. It was found that, during this period, the progression rate of some Osteoarthritis assessments, including OARSI score, cartilage area and synovitis score, did not change significantly after metformin administration; in contrast, the progression rate of some other Osteoarthritis assessments, including osteophyte size and osteophyte maturity was significantly reduced after metformin administration. In this study, the knee joint phenotype induced by DMM surgery in AMPKα1 KO mice was not significantly different from that of WT mice; however, metformin had a protective effect on the Osteoarthritis progression of WT mice, but not that of AMPKα1 KO mice. These results suggest that normal AMPKα1 levels may not be required to maintain joint tissue homeostasis and metformin does play a chondroprotective effect through activation of AMPK signaling. The AMPKα1 KO mice used were global AMPKα1 KO mice, not chondrocyte-specific KO mice as in previous studies. In this in vitro study, metformin downregulated pAMPKα1 expression in a short period of time. In contrast, metformin significantly increased total AMPKα1 expression in articular chondrocytes and in DRG cells. This difference may be due to the duration of treatment. In vivo treatment with metformin was 6 and 12 weeks. AMPK activation plays an important role in blocking pain sensitivity. Pain hypersensitivity due to injury or disease is often aligned with increased excitability of peripheral sensory neurons in the DRG. TRPA1 channels are widely recognized chemical and thermal sensors that play an important role in pain transduction. Metformin, as an AMPK activator, inhibited TRPA1 activity in DRG neurons by inhibiting TRPA1 expression and metformin also inhibited TRPA1-mediated calcium influx. From the limited-term study, the pain-relieving effect of metformin may be related to its chondroprotective effect on DMM-induced Osteoarthritis pathology.

In addition to DMM which is a surgical induction, there are also other methods to induce Osteoarthritis, namely injection induction using Monosodium acetate (MIA). The study of Na et al found that metformin reduced pain in MIA-induced Osteoarthritis rats. The ability of metformin to reduce pain in MIA-induced Osteoarthritis rats was assessed based on secondary tactile allodynia. Compared with MIA control rats, metformin administration increased paw-withdrawal latency (PWL), paw-withdrawal threshold (PWT), and weight-bearing. In addition, CGRP expression in the dorsal root ganglia (DRG) decreased in the metformin-treated group compared with the control group, but not in TRPV1. These results imply that metformin may alleviate Osteoarthritis-related pain.

Immunohistochemistry was used to detect inflammatory mediators and catabolic factor production in Osteoarthritis rat joints. In the metformin-treated group, the expression of MMP-3, IL-1P, iNOS, and IL-17 was reduced in Osteoarthritis synovium tissues. These results imply that metformin limits catabolic and inflammatory responses in MIA-induced Osteoarthritis rats.

Immunohistochemistry was used to detect autophagy and cell-death mediator levels in MIA-treated rat joints. AMPK expression was significantly increased by metformin administration, whereas caspase-1 and p-MLKL levels were significantly reduced. Western blotting revealed increased expression of caspase-3, p-AMPK, LC32b, and p62, while caspase-1 decreased in chondrocytes treated with metformin. In addition, the death factor caspase-1, increased with hydroxychloroquine (HCQ) treatment, which is an autophagy inhibitor. These results suggest that metformin regulates inflammatory cell death through inhibition of autophagy in osteoarthritis chondrocytes.

Celecoxib has been known to have therapeutic effects on Osteoarthritis. The damaged cartilage showed dramatic improvement in the group treated with the combination of metformin and celecoxib. These results demonstrate the therapeutic potential of metformin and celecoxib combination treatment.

## III. CONCLUSION

Based on the results of the data and discussion that has been done, it can be concluded that the administration of metformin has an influence on the progressivity of osteoarthritis in experimental rat animals, and the OARSI score in rat animals given metformin intervention shows better results than in experimental rat animals that are not intervened with metformin.

## IV. ACKNOWLEDGMENT

Author would like to thank Rosavelina Sintaasih Budihardjo, Razita Aulia Azkia, Naoval Diza Ananda, Selvia Yuliani Dwi Ratih Sanggarwati for the participation to make this review study possible.

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