International Summit on Science Technology and Humanity (ISETH2019)
Advancing Scientific Thought for Future Sustainable Development
p-ISSN: 2477-3328
e-ISSN: 2615-1588

Effect of Green Tea Extract to the Degree of Knee Joint Damage and Nitric Oxide Levels in the Rabbit Osteoarthitis Model

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Abstract

Osteoathritis (OA) is characterized by degeneration of articular cartilage, subchondral bone, synovial fluid and synovium. Nitric oxide (NO) is proinflammatory cytokine that play a significant role in the pathogenesis of OA via cartilage and bone degradation by synovial inflammation. Green tea is a novel functional food for treating osteoarthritis and inhibiting the production of inflammatory mediators such as nitric oxide. This study aimed to evaluate the effects of green tea extract to the nitric oxide levels and degree of knee joint damage in the rabbit osteoarthitis model. The Freud's adjuvant complete was performed to induce OA, as many as sixteen male rabbits (New Zealand white) were randomly divided into four groups: adjuvant injection, adjuvant and green tea (injection), adjuvant and green tea (per oral), and control group. The control group only received drinking water, the Freud adjuvant (0.2 ml) and green tea extract (200 mg/kg bw) were orally and injection administered for eight weeks. The articular cartilage damage was evaluated histologically according to MANKIN score. NO levels were determined by nitric oxide assay. Data was analysed by Chi square test. The result of this study showed the surface structure damage of cartilage increased after adjuvant-induced. Green tea extract decrease significantly (p=0.02) the degree of knee joint damage after adjuvant-induced in rabbit osteoarthritis models. NO levels increased after OA induction. The green tea extract administration (via injection) can significantly (p = 0.038) decrease NO levels compare to adjuvant group. Green tea extract decrease the knee joint damage and NO proinflamantory levels in rabbit of osteoarthritis model

Keyword: MANKIN score, green tea, nitric oxide, osteoarthritis

INTRODUCTION

In the world, incidence of age-related diseases of muscle, and bone joint is seriously affecting the health of millions of people. According to the World Health Organization (WHO), arthritic, musculoskeletal and rematic conditions are leading causes of morbidity and disability. The most common form of arthritis is osteoarthritis (OA) (osteoarthrosis or degenerative joint disease). OA is the most common type of degenerative joint disease. Osteoarthritis (OA) is characterized by degeneration of articular cartilage, changes in subchondral bone integrity, osteophyte formation, inflammation of the synovium tissue and tendon, and muscle weakness (Li et al., 2013). The major mediators of inflammatory responses in OA are proinflammatory cytokines. These proteins are involved in the pathogenesis of OA that cause the cartilage and bone changes and synovial during disease progression. Proinflammatory mediators (nitric oxide/NO, cytokines, neuropeptides, and prostaglandin are produced by alter the balance of cartilage matrix degradation and the inflamed synovium that excess production of the proteolytic enzymes for cartilage breakdown. Cartilage alterations induce synovial inflammation, then exacerbate clinical symptoms and joint degradation in OA (Rainbow et. al., 2012).

Osteoarthritis (OA) is a degenerative disease involving chondrocytes, cartilage and other joint tissues, and has a number of underlying causes, including both biochemical and mechanical

factors. Despite the worldwide prevalence of OA, there are still questions about the events that cause OA, making it difficult to identify potential disease-modifying targets. The pathogenesis of disease, particularly early disease, is still not completely understood. As a result, development of effective tools for early diagnosis and disease-modifying therapeutics has been hampered. For the evaluation of osteoarthritic (OA) cartilage, the histologic/histochemical grading of MANKIN system has been widely used. This system was developed for the assessment of human hip OA cartilage and to evaluate cartilage degradation, repair and regeneration in osteoarthritis animal models. The assessment of the MANKIN system includes four parameters, namely cartilage structure, cellularity, tidemark integrity and Safranin O staining. Each parameter has subcategories and the scores are summed to provide a total score (Pauli et al., 2012)

Proinflammatory mediators, including nitric oxide (NO), IL-1, tumor necrosis factor (TNF)-α, and prostaglandins, are all over-produced in chondrocytes. Although proinflammatory factors including nitric oxide (NO) are associated with OA, there is recent evidence suggesting that NO and its redox derivatives may also play protective roles in the joint. Experiments have demonstrated that NO plays a catabolic role in the development of OA and mediates the inflammatory response, is involved in the degradation of matrix metalloproteinases, inhibits the synthesis of both collagen and proteoglycans, and helps to mediate apoptosis (Lana and Rodrigues, 2019).

Nitric oxide (NO) is a predominant mediator in progression of OA and chondrocyte apoptosis. The previous studies indicated that increased concentration of nitrite (a NO metabolite) in serum and synovial fluid samples of patients with rheumatoid arthritis and OA and have therefore suggested a role for NO as an inflammatory mediator in rheumatic diseases (Hsu et al. 2017)· However, the exact mechanism in which NO regulates the immune system in OA chondrocytes remains unclear.

Commonly prescribed analgesics and nonsteroidal anti-inflammatory drugs (NSAIDs) provide symptomatic relief but do not have any demonstrated any beneficial effect on OA disease prevention or modification (Le Graverand-Gastineau, 2010). Furthermore, long-term use of these drugs has in some cases been associated with substantial gastrointestinal, renal and cardiovascular side effects. Because the nature of OA likely requires decades-long treatment (Martel-Pelletier et al., 2012), novel therapies to combat this disease must be safe for clinical use over long periods of time.

Green tea is produced from fresh leaves in such a way that prevents oxidation of polyphenolic components (mainly catechins), oolong tea polyphenols are partially oxidized, while polyphenols in black tea undergo a high degree of oxidation. Components of green tea beverage measured as weight percentage of extract solids include 30%–42% catechins, 5%–10% flavonols, and 2%–4% other flavonoids. According to the United StatesDepartment of Agriculture (USDA) Flavonoid Database, brewed greentea contains an average of 126.6 mg total catechins and 77.8 mg EGCG per 100 ml as consumed, on the basis of 1 g leaf/100 mL infusion.Consequently, each 240 mL serving of brewed green tea may provide anestimated 304 mg total catechins, with 187 mg EGCG. (Graham, 1992; Hu et al., 2018). Catechins are divided into catechin (C), (–)-epicatechin (EC), (–)-epicatechin gallate (ECG), (–)-epigallocatechin (EGC), and (–)-epigallocatechin-3-gallate (EGCG)

The levels of polyphenol content vary according to type of tea, brewing, commercial brand, and producing country, the polyphenol contents of black and green tea leaves to be 80.5 to 134.9 mg/g and 65.8 to 106.2 mg/g, respectively (Khokhar and Magnusdottir, 2002). Green tea extract showed high antioxidant activities against DPPH and NBT radicals (96.5 and 89.6% for DPPH and NBT

p-ISSN: 2477-3328
e-ISSN: 2615-1588

free radicals, respectively) at a concentration of 64 μg ml^{-1} (Alghadir, et al., 2016). These results proved previous research works that reported high antioxidant activity of green tea extracts.

Epigallocatechin 3-gallate (EGCG), a major bioactive polyphenol present in green tea, belongs to a group of food-derived products, termed nutraceuticals, with re- ported health benefits. Nutraceuticals have been sug- gested as safe alternatives or supplements to current pharmacologic therapies for OA (Olsen, 2011; Akhtar and Haqqi, 2012) EGCG exerts numerous health-promoting effects to counteract inflammation, aging and cancer (Akhtar and Haqqi, 2012). In addition, EGCG has other reported effects particularly relevant to OA, such as inhibiting the production of inflammatory mediators such as nitric oxide, prostaglandin E_2 (PGE_2), cyclooxygenase-2 (COX-2), inducible nitric oxide synthase and interleukin (IL)-8 in human and equine chondrocytes in vitro (Heinecke et al., 2010). EGCG, the major and most active component of GTPs, protects human chondrocytes from IL-1β-induced inflammatory responses, and suggests the potential of EGCG in OA treatment/prevention.

EGCG has other reported effects particularly relevant to OA, such as inhibiting the production of inflammatory mediators such as nitric oxide, prostaglandin E_2 (PGE_2), cyclooxygenase-2 (COX-2), inducible nitric oxide synthase and interleukin (IL)-8 in human and equine chondrocytes *in vitro* (Heinecke et al.2010). EGCG inhibits protein expression of matrix metalloproteinase (MMP-1 and MMP-13), its also suppresses IL-1β-induced glycosaminoglycan release from cartilage by reducing a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS1, ADAMTS4 and ADAMTS5 (Leong et al. 2014).

Polyphenols are antioxidants and play a role in preventing oxidative damage caused by the reactive oxygen species (ROS) (Kun Li et al., 2016). They possess anti-inflammatory and anti-carcinogenic properties and may aid in the prevention of cardiovascular disease. Certain pathways have been proposed to support the protective effect of green tea against arthritis, the most popular of these pathways previously reported, are the ability of green tea to regulate the endocrine system, redox status, and regulation of TNF–α gene expression, which suppresses the release of TNF-α, IL-1, and IL-6 (Feldmann and Maini, 2008). These proinflammatory cytokines play a significant role in the pathogenesis of OA and RA via cartilage and bone degradation by synovial inflammation. Green tea polyphenols added to drinking water reduce the incidence of collagen-induced arthritis and decrease the tumor necrosis factor (TNF)-α and COX-2 levels in articular joints in mice (Haqqi et al., 1999). However, the extent to which EGCG alters OA progression *in vivo* and improves OA-related symptoms, especially pain, has not been reported. Thus, green tea preserves the integrity of cartilage and prevents the release of cartilage and collagen degradation markers, which increase during progressive cartilage degradation in OA and RA (Alghadir, et al., 2016; Gabr et al., 2014).

Currently, most pharmacologic treatments are concentrated on secondary effects of the disease, such as relieving pain and improving joint function, but fail to address the evolving and complex nature of OA (Kon et. al., 2012). Commonly prescribed analgesics and nonsteroidal anti-inflammatory drugs (NSAIDs) provide symptomatic relief but do not have any demonstrated any beneficial effect on OA disease prevention or modification (Lapane et al., 2015). Furthermore, long-term use of these drugs has in some cases been associated with substantial gastrointestinal, renal and cardiovascular side effects (Martel-Pelletier, 2012). Because the nature of OA likely requires decades-long treatment (Le Graverand-Gastineau , 2010), novel therapies to combat this disease must be safe for clinical use over long periods of time.

There is no effective pharmacotherapy capable of restoring structure and function of damage of the cartilage and synovial tissues, side-effects of pharmaceutical treatment, limitations to conventional medical, effective, and safe for OA patients so that herbal medicines have the

potential to provide a solution to this problem. Thus new strategies based of non drug treatment such as physical activity and exercise, with natural plant products have received attention in the treatment of OA. Therefore, this study aimed to evaluate the effects of green tea extract to the nitric oxide level and degree of knee joint damage in the rabbit osteoarthitis model

METHODS

Green tea leaves is obtained from the South Bandung Tea Plantation. Green tea leaf extraction was carried out by maceration method. This study was an experimental study by using New Zealand white rabbits (Oryctolagus cuniculus), male, with age and weight that are not much different. Rabbits with an adult bone growth period, which is above 2 months.

The Freud's adjuvant complete was performed to induce OA, 16 male New Zealand white rabbits were randomly divided into four groups: adjuvant group, adjuvant and green tea (injection) group, adjuvant and green tea (orally) group, and control group. The control group received drinking water, the Freud's adjuvant complete and green tea extract groups were orally and injection administered with 200 mg/kg of green tea extract and 0.2 ml of Freud's adjuvant for eight weeks.

The articular cartilage damage was evaluated histologically according to MANKIN score. A partial section of surface structure abnormalities was allocated. The evaluation was performed at the weight bearing surface structure of the medial femoral condyle because it showed most severe histological abnormalities and was the earliest structure formed. To make histological preparations, the knee bone was decalcified in 10% citric acid for 3x24 hours. The bone is cut longitudinally and put in a paraffin block for 24 hours. The histological preparations were stained with hematoxylin eosin

NO levels were determined by Nitric Oxide Assay Kit (colorimetric) (abcam 65328) The first step converts nitrate to nitrite utilizing nitrate reductase. The second step uses Griess Reagents to convert nitrite to a deep purple azo compound. The amount of the azochromophore accurately reflects nitric oxide amount in samples. NO is rapidly oxidized to nitrite and nitrate which are used to quantitate NO production. Data was analysed by Chi square test. We acquired approval by Use Committee of YARSI University (ethics code: 113/KEP-UY/BIA/VI/2019) for experimental procedures.

RESULTS

In this study, the histological assessment of the knee joint cartilage was guided by MANKIN parameters (Figure 1). The parameter of assessment was the surface structure of the knee joint cartilage. The results showed that the surface structure of the cartilage of adjuvant-induced rabbits suffered damage with uneven surfaces, forming undulations and irregulations (Figure 2) with the degree of surface structure damage is 2.5. After administration of the green tea extract showed a repair that significantly ($p = 0.02$) decreased the degree of knee joint damage to 1.5 (injection) and 1.8 (orally) (Figure 3).

p-ISSN: 2477-3328
e-ISSN: 2615-1588

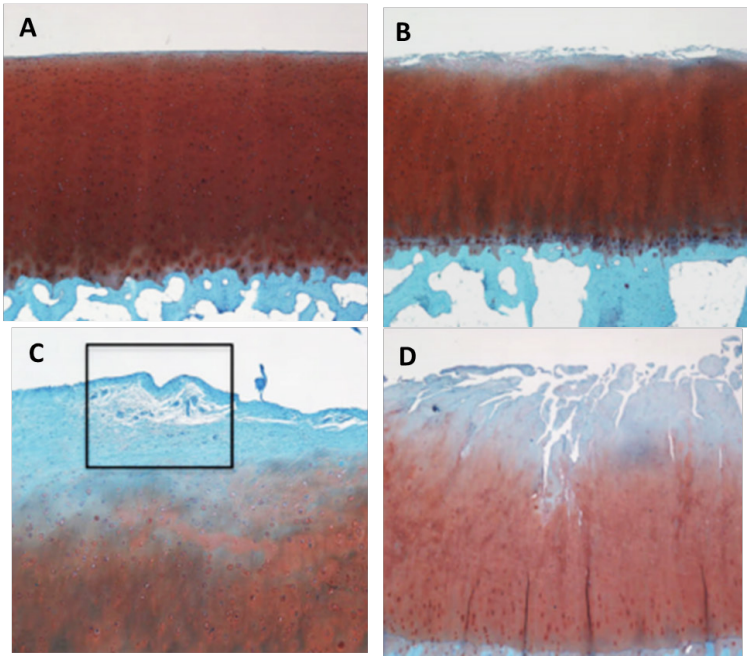

Figure 1. Histopatological assessment of the surface structure from femoral condyles sections according to MANKIN's grading. A) Normal (intact smooth surface), grade 0. B) Surface irregularities/undulations, grade 1. C) surface irregularities (fibrillation) and pannus, grade 2. D) transitional zone clefted, grade 3. magnif.40x (Pauli et al. 2012).

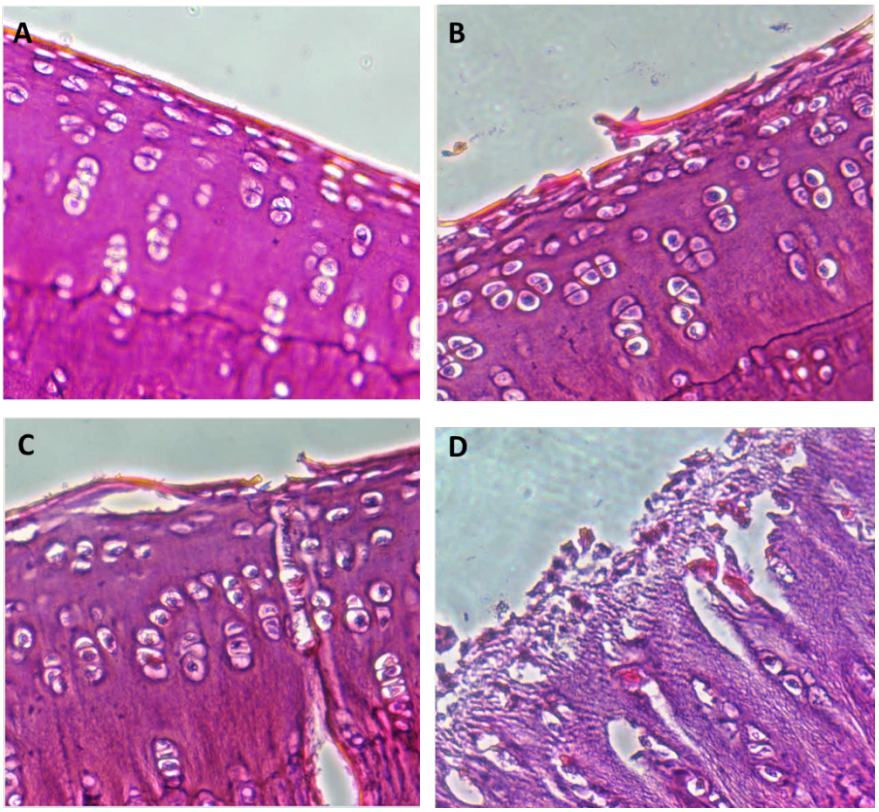

Figure 2. Histological comparison of the surface structure of the knee joint based on Mankin's score. A) grade 0. B) grade 1. C) grade 2. D) grade 3. Magnification 200x.

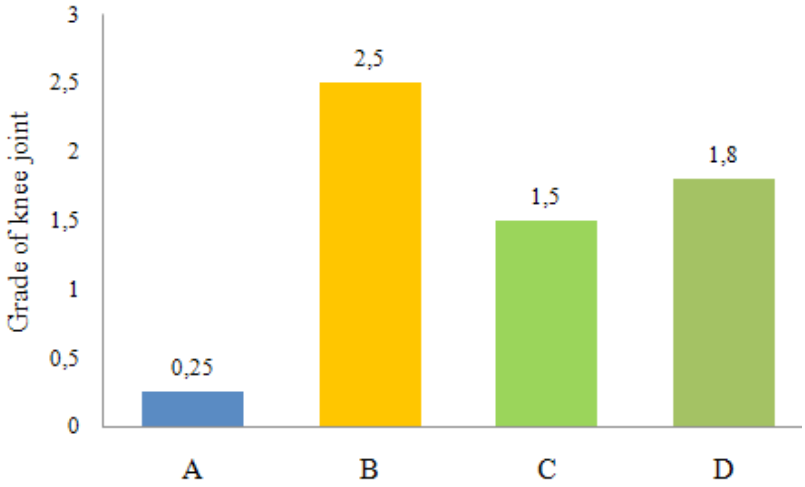

Figure 3. Diagram of knee joint degree in rabbit osteoarthritis model. A) Control, B) freud adjuvant, C) freud adjuvant and green tea extract (injection), and D) Freud adjuvant and green tea (per oral)

The result of study also showed that the rabbit osteoarthritis model injected adjuvant increase NO levels compared to controls. Administration of green tea extract in rabbit osteoarthritis models can significantly decrease NO levels ($p = 0.038$; $p<0.05$). The injection administration is more effective compare to per oral in rabbit osteoatritis models (Figure 4.)

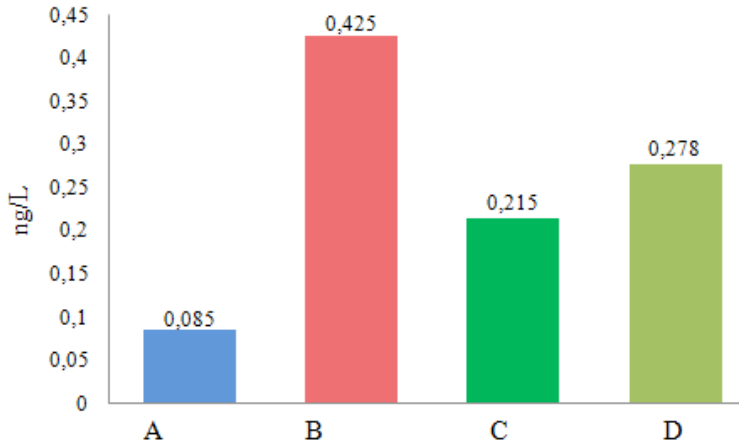

Figure 4. Nitric oxide level in knee joint of rabbit osteoarthritis model. A) Control, B) freud adjuvant, C) freud adjuvant and green tea extract (injection), and D) Freud adjuvant and green tea (per oral)

DISCUSSION

Osteoarthritis (OA), as a degenerative joint disease is frequently seen in the elderly population (Appleton, 2018). The pathological hallmark of OA development is cartilage hypertrophy, synovitis the articular cartilage loss, and subchondral bone recasting (Glyn-Jones et al., 2015; Flemming and Gustas-French, 2017; Scotece et al., 2018). The pathophysiology of OA is not yet fully understood, inflammation-related biomarkers and inflammantory seem to play a critical part (Jotanovic et al., 2012).

p-ISSN: 2477-3328
e-ISSN: 2615-1588

The main initiators in the development of OA, exerts harmful effects by up-regulating the production of catabolic factors and pro-inflammatory factors including nitric oxide (NO), matrix metalloproteinases (MMPs), prostaglandin E_2 (PGE_2), and thrombospondin motifs (ADAMTS), which ultimately lead to the extracellular matrix (ECM) degradation (Verma and Dalal, 2011; Zeng et al., 2015; Zhou et al., 2018).

The results of this study shows that based on Mankin score, adjuvant-induced can damage the cartilage surface structure of knee joint in osteoarthritis rabbits model The Mankin system describes features such as surface irregularities and complete disorganization. These two parameters as critical for the assessment of cartilage degradation, surface irregularities may also be found in healthy cartilage and lead to a lower score (Rutgers et al., 2012). Adjuvant, an inflammatory mediator in osteoatritis,cause alter the balance of cartilage matrix degradation. This result of study reported green tea extract repaired the cartilage degradation and surface irregularities, which is caused by EGCG activity in green tea. The results of this study support previous studies of EGCG activity.The chondroprotective effects of EGCG on attenuating inflammation have been well established in rheumatoid arthritis animal models, studies using human and bovine cartilage explants, synovial fibroblasts, human chondrocytes (Akhtar and Haqqi, 2014; Leong t al., 2014). EGCG was nontoxic to human chondrocytes (Singh et al., 2002).

In humans, daily doses of 800 mg of EGCG for 4 weeks are safe and well tolerated. EGCG is mostly absorbed by the small intestine and may undergo gastrointestinal inactivation. Therefore, oral administration of EGCG may reduce its bioavailability, administer EGCG systemically via intraperitoneal injection, leads to higher bioavailability compared to oral consumption (Mereles and Hunstein, 2011). The results of this study shows administration of the green tea extract via intraperitoneal injection more effective repairs knee joint compared to oral consumption.

This study also proved that the osteoarthritis rabbits model were injected adjuvant increasing in NO levels compared to controls. Administration of green tea extract in osteoarthritis rabbit model can decrease NO levels. This study also proved that the rabbit model of adjuvant-induced osteoarthritis increased NO levels compared to controls. The rabbit osteoarthritis models given green leaf extract reduced NO levels. This result supports previous research that there was an increase of NO concentrations in adjuvant-induced arthritis rats and arthritis patients. These patients were found to have elevated levels of NO in serum and synovial fluid compared to normal controls. Increased of levels NO level have been found in the urine and plasma of adjuvant-induced mice. In the pathophysiology of osteoathritis, NO involvement may be a potential target for osteoathritis. Decreased of NO levels occur due to EGCG activity in green tea catechins which inhibits NO production (Heinecke et al. 2010) . The possible mechanism of green tea extract activity may be due to free radical scavenging potential caused by the presence of catechin antioxidant components.

Nitric oxide (NO) has been definitively recognised as one of the key players involved in immunity and inflammation, as an important chemical mediator of inflammation. Osteoarthritic chondrocytes produce a number of inflammatory mediators including NO, IL-1, TNF, and prostaglandins. NO is expressed in human osteoarthritis cartilage, mediates the expression of proinflammatory cytokines, inhibited the synthesis of collagen and proteoglycans and inducing apoptosis, activates metalloproteinases, and promotes chondrocyte inflammatory responses. The inducible nitric oxide synthase (iNOS) enzyme is also upregulated in OA chondrocytes, resulting in an excess of NO and release of inflammatory cytokines (Abramson, 2008). Nitric oxide (NO) synthesized from arginine by nitric oxide synthases (NOS). The inducible isoform of NOS (iNOS) produced large amounts of NO, its overexpression was linked to the tissue destruction and progressive inflammation in human rheumatoid synovium and hypoxic experimental arthritis

(Ahn et al. 2008; Farel et al., 1992; Chou, 2011). Cartliage may be protected by NO regulating the balance between MMP and TIMP, both of which are involved in the pathogenesis of cartilage breakdown. Increased accumulation of NO accompanied with decreased MMPs and increased TIMP-2 expression (Hsu et al., 2017).

CONCLUSION

Green tea extract has a protective effect on the surface structure degradation of the knee cartilage in osteoarthritis rabbit model through a decrease in nitric oxide levels.

ACKNOWLEDGMENT

This research has been funded by the research grant of the Ministry of Research, Technology and Higher Education, Indonesia with Contract no .32/AKM/PNT/2019.

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p-ISSN: 2477-3328
e-ISSN: 2615-1588

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