

# JOURNAL OF BIOMEDICINE AND TRANSLATIONAL RESEARCH

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Research Articles

## Peanut (*Arachis hypogaea*) Shells Extract and *Apis dorsata* Honey Reduce Matrix Metalloproteinase-3 in Monosodium Iodoacetate-Induced Osteoarthritic Rats

Bella Stevanny<sup>1</sup>, Tungki Pratama Umar<sup>1\*</sup>, Junoretta Haviva Ernanto<sup>2</sup>, Rachmat Hidayat<sup>3</sup>

<sup>1</sup>Medical Profession Program, Faculty of Medicine, Sriwijaya University, Indonesia

<sup>2</sup>Medical Education Program, Faculty of Medicine, Sriwijaya University, Indonesia

<sup>3</sup>Department of Biology, Faculty of Medicine, Sriwijaya University, Indonesia

### Article Info

History

Received: 27 May 2020

Accepted: 19 Nov 2020

Available: 31 Dec 2020

### Abstract

**Background:** Osteoarthritis is the most common degenerative joint disease worldwide, but its treatment can cause serious adverse events. Ethanolic extract of peanut shells contains luteolin functioned as its primary anti-inflammatory and chondroprotective agent. *Apis dorsata* honey also contains anti-inflammatory and antioxidant agents, especially from the *Tualang* honey type. Both have the potential to reduce inflammation and prevent articular degradation in osteoarthritis.

**Objective:** This study aims to determine the effect of peanut shells extract and *Apis dorsata* honey on matrix metalloproteinase-3 (MMP-3) level in monosodium iodoacetate (MIA)-induced osteoarthritic rats.

**Methods:** In this in vivo study, female Wistar rats (n=27) were randomly divided into nine groups containing three rats each. Treatment was given to group 1, 2, and 3: Tualang honey (TH) 25% + peanut shells extract (PSE) with 1%, 5%, and 10% concentration; group 4, 5, and 6: TH 50% + PSE with 1%, 5%, and 10% concentration; group 7: diclofenac sodium (positive control); group 8: aquadest (negative control); and group 9: aquadest (normal) for 10 days. Knee osteoarthritis was induced by intra-articular injection of MIA on day 4. Anti-inflammatory and chondroprotective activities were evaluated with MMP-3 ELISA.

**Results:** The mixture of peanut shells extract and *Apis dorsata* honey significantly reduced MMP-3 level in group 1 (331.12 pg/ml), group 2 (291.73 pg/ml), group 3 (266.58 pg/ml), group 4 (274.15 pg/ml), group 5 (251.12 pg/ml), and group 6 (220.52 pg/ml) after 10 days of treatment. The MMP-3 level was also evaluated in group 7 (169.61 pg/ml), group 8 (413.55 pg/ml), and group 9 (39 pg/ml). Compared to the negative control group, treatment and diclofenac groups significantly reduced the MMP-3 level in patello-femoral articular cartilage.

**Conclusion:** Peanut shells extract and *Apis dorsata* honey showed an anti-inflammatory and chondroprotective effect by reducing the MMP-3 level in MIA-induced osteoarthritic rats.

### Keyword:

*Apis dorsata* honey; Inflammation; MMP-3; Osteoarthritis; Peanut shells extract

**Permalink/ DOI:** <https://doi.org/10.14710/jbtr.v6i3.7908>

### INTRODUCTION

Osteoarthritis (also known as OA) is the most common degenerative joint disease worldwide. OA is affiliated with damage to articular cartilage in relation to complex interactions between genetic, metabolic, biochemical, and biomechanical factors. All of these factors

contribute to the inflammatory response involving subchondral bone, cartilage, and synovium.<sup>1</sup>

\*Corresponding author:

Email: [tungkipratama@gmail.com](mailto:tungkipratama@gmail.com)  
(Tungki Pratama Umar)

This condition is affecting about 242 million people worldwide, commonly in the hip or knee joint. Pathophysiology of this condition is characterized by destruction of articular cartilage, inflammation of the synovial membrane, and subchondral bone remodeling.<sup>2,3</sup>

Almost all patients with OA complain about pain and stiffness as fundamental causes of burden of their quality of life. Therefore, the treatment principles of OA are to reduce pain, relieve stiffness, and maintain joint function. Current guidelines for OA treatment recommend the use of combination therapy consisting of non-pharmacological (i.e., physical therapy), pharmacological (non-steroid anti-inflammatory drugs), and surgical intervention if needed. Despite its rapid, short-term analgesic and anti-inflammatory effects, prolonged use of non-steroid anti-inflammatory drugs (NSAID) can cause serious adverse events, including toxicity and increased risk of gastrointestinal bleeding.<sup>3,4</sup> Over the last few decades, researchers have improved understanding of the pathophysiology of the disease. Osteoarthritis is generally defined as a chronic inflammatory disease with a gradual decline of the immune system causing the decline of quality of life. Therefore, the discovery of agents to prevent or slow the development of this degenerative inflammatory disease is of great importance. Since ancient times, Chinese and Indian (Ayurvedic) medicines have used herbs to treat numerous diseases. Herbal medicine is believed to have fewer side effects compared to the synthetic chemical drug. Furthermore, most of the herbal medicines have antioxidant properties regulating oxidative metabolism to prevent or reduce oxidative stress.<sup>5</sup> Peanut shells (*Arachis hypogaea*) are commonly known as an agricultural waste of low economic value but have a good source of natural antioxidants, including polyphenol (gallic acid) and flavonoid (quercetin).<sup>6</sup> Peanut shells have the potential to reduce inflammation and prevent articular cartilage degradation. Ethanol extract of peanut shells contains luteolin as its major flavonoid content functioned as its primary anti-inflammatory and chondroprotective agent.<sup>7</sup>

Tualang honey (TH) is produced by rock bees (*Apis dorsata*) and is known for its antioxidant, anti-inflammatory, antimicrobial, and anti-mutagenic effects. TH contains higher flavonoids, phenolics, and 5-(hydroxymethyl) furfural (HMF) compared to most types of honey, including Manuka honey. The antioxidant can suppress oxidative stress that occurs in degenerative diseases.<sup>8</sup>

The combination of peanut shells and Tualang honey might potentially be a novel alternative anti-inflammatory and chondroprotective agent for patients with OA with minimal side effects in long-term use. Matrix metalloproteinase-3 is mainly found in the synovium, articular cartilage surface, and pannus-like tissue in osteoarthritis. This study aims to determine the effect of peanut shells extract and *Apis dorsata* honey on matrix metalloproteinase-3 (MMP-3) level in monosodium iodoacetate (MIA) induced osteoarthritic rats.

## MATERIALS AND METHODS

### Preparation of Peanut Shells Extract and Phytochemical Test

*Arachis hypogaea* shells were collected from the Indonesian Center for Research and Development of Medicinal and Traditional Medicinal Plants (*Balai Besar*

*Penelitian dan Pengembangan Tanaman Obat dan Obat Tradisional, B2P2TOOT*) in Tawangmangu, Karanganyar, Central Java, Indonesia. The dried shell was then mashed up to obtain the fine powder. A total of 1 kg of fine powder was obtained. Maceration was conducted in 96% ethanol (Merck®) at the ratio of solvent:dried shell (10:1) for 3x24 hours at room temperature.<sup>9</sup> Homogenization was maintained by stirring the mixture for every 12 hours. The macerate was then filtered using filtration paper to obtain two parts: “residual part” and “filtered part”.<sup>10</sup> The filtered part was then evaporated using a rotary vacuum evaporator (Heidolph®) at 175 mbar and vapor temperature of 60°C until a constant weight was obtained.<sup>9</sup> The concentrated extracts were then dried and gathered. After the extract was weighed, it was stored in a sealed, air-tight container, under dark condition at room temperature.<sup>11</sup> Finally, phytochemical test was done to determine the content of flavonoids (Dragendorff’s test, Mayer’s test), steroids and triterpenoids (Liebermann–Burchard test), alkaloids (Wagner’s test), tannins (Ferric chloride test), saponins (Foam test), and quinones (Ammonium hydroxide test).<sup>12,13</sup>

### Preparation of Syrup Made of Peanut Shells Extract and *Apis dorsata* Honey

Peanut shells extract (PSE) was dissolved in 1% Carboxymethylcellulose (CMC, Merck®). The remaining materials (TH, nipagin, propylene glycol) were then added. Diclofenac sodium was also dissolved the same way. Finally, the aquadest was added to obtain the total syrup volume of 42 mL.

### Experimental Animals

Twenty-seven 20-week-old female Wistar white rats (*Rattus norvegicus*) with a mean bodyweight of 200-300 grams were collected from the Eureka Research Laboratory, Palembang, Indonesia. This study was conducted after the approval by the Research Ethics Committee of the Sriwijaya University Faculty of Medicine, Palembang, Indonesia. The animals were housed in plastic cages and had *ad libitum* access to water and standard feeding.<sup>14</sup> Acclimatization was done for seven days before the experimental procedure, at the temperature of about 22°C, atmospheric humidity (50-60%), and light-dark cycle (12h/12h) were maintained in the room.

### Osteoarthritis Induction

Rats were anesthetized with ketamine-xylazine-acetylpromazine (KXA, Sigma-Aldrich®) 0,1 mL/10gBW intraperitoneally. In accordance with Takashi *et al.*<sup>15</sup>, OA was induced by injecting monosodium iodoacetate (MIA, Sigma-Aldrich®) at a dose of 1 mg/0,1mL normal saline to the intra-articular space of the right knee joint.

### Experimental Protocol

Twenty-seven female Wistar rats were randomly assigned into nine groups with three rats in each group. Group 1: treated with TH 25% + PSE 1%, group 2: treated with TH 25% + PSE 5%, group 3: treated with TH 25% + PSE 10%, group 4: treated with TH 50% + PSE 1%, group 5: treated with TH 50% + PSE 5%, group 6: treated with TH 50% + PSE 10%, group 7: treated with diclofenac sodium (positive control); group 8: negative

control; and group 9: normal. All treatments were given per oral as much as 1 mL for ten days.

### Matrix Metalloproteinase (MMP-3) Level Evaluation

Chloroform (Merck®) was used to euthanize rats by inhalation. Patellofemoral articular cartilage tissue is then evacuated and placed in microtubes. Phosphate-buffered saline (PBS, Sigma®) is added. The tissue was homogenized with a microtube pestle on top of the vortex mixer, and then centrifugated (2000-3000 rpm, 4°C) for 20 minutes. The supernatant was used as ELISA test sample. The assay was done using Rat MMP-3 ELISA Kit (CloudClone®). The optical density (OD) was measured using an ELISA reader.<sup>16</sup> Microsoft® excel software was then used to collect data and calculate MMP-3 expression.

### Statistical Analysis

Matrix metalloproteinase-3 level data were evaluated using the IBM® Statistical Package for the Social Sciences (SPSS®) 25.0. Shapiro Wilk normality test is used to know the normality of the data distribution, followed by Levene homogeneity test. One-way ANOVA and post hoc Tukey tests were then performed to determine which group have significant differences. The significance is set at  $p$  value < 0.05.

## RESULTS

### Phytochemical Screening

Flavonoids and quinones were found positive in our *Arachis hypogaea* shells extract. In addition, alkaloids, tannins, and triterpenoids were also present in a lesser amount compared to flavonoids and quinones. Ad interim, steroids as well as saponins were not found. Phytochemical screening results are displayed in Table 1.

**Table 1.** Phytochemical screening of *Arachis hypogaea* Shells Extract

Tests	Results
Flavonoids	+
Alkaloids	+
Triterpenoids	+
Steroids	-
Saponins	-
Tannins	+
Quinones	+

### MMP-3 Level in Patellofemoral Articular Cartilage

The effect of treatment using the different doses of PSE and TH on MMP-3 expression in rat patello-femoral articular cartilage tissues were shown in Figure 1. All treatment groups, which were given the combination of PSE and TH, showed significantly different results ( $p < 0,05$ ) compared to the negative control group. The best result can be seen in the sixth treatment group, which was given the highest doses (50% TH and 10% PSE). Its effect showed a dose-dependent manner.

## DISCUSSION

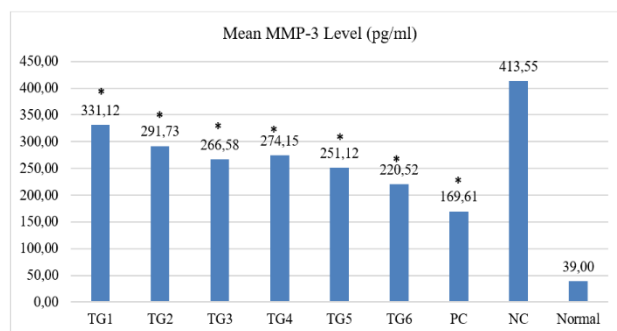
Osteoarthritis pathogenesis is based on biochemical and biomechanical factors which cause an imbalance between articular cartilage formation and degradation.<sup>17</sup> Osteoarthritis is associated with the activation of matrix

metalloproteinase (MMP) enzymes responsible for proteoglycan and collagen degradation in articular cartilage.<sup>18</sup> MMP-3 is one of the MMPs responsible for procollagenase activation and proteoglycan degradation in the joint.<sup>19</sup> The MMP-3 activity was exhibited to disintegrate some proteins on extracellular matrix (ECM), such as fibronectin and laminin, and also related to the activation of MMP-13 and gelatinases cascade.<sup>20</sup> Expression of MMP-3 is mainly found in the synovium, articular cartilage surface, and pannus-like tissue in osteoarthritis patients and therefore considered as a potential biomarker for knee osteoarthritis.<sup>21,22</sup>

The administration of *Arachis hypogaea* (peanut) shells extract and honey combination syrup can decrease the inflammatory process in osteoarthritis. In previous study, this effect is based on its phytochemical compounds: phenolic compounds, alkaloids, and saponins. These phenolic compounds have redox characteristic which is aimed at their antioxidative capacity. Alkaloids such as quinolones have an antioxidative effect. Saponins can act as transition metal ( $\text{Cu}^{2+}$  or  $\text{Fe}^{2+}$ ) and decrease cellular sensitivity to oxidative stress.<sup>23</sup> In our study, it shows a dose-dependent manner of therapeutic effects, as illustrated in Figure 1. Its increment of effect also showed relatively similar values with increasing material percentage.

Peanut (*Arachis hypogaea*) shells contain luteolin as their main phenolic compound from flavonoid group,<sup>24,25</sup> which can inhibit MMP-3 gene expression in IL-1 $\beta$ -induced chondrocytes. This shows luteolin's capability to inhibit protein synthesis, in this case, MMP-3 secretion in chondrocyte, as well as its chondroprotective activity.<sup>26</sup> Aside from flavonoids, phytochemical tests also showed that peanut shells extract also contains quinones, alkaloids, triterpenoids, and tannins. These contents may increase the anti-inflammatory and antioxidant effects of the extract. *Apis dorsata* or *Tualang* honey, on the other hand, contains flavonoids, alkaloids, saponins, and triterpenoids. Its flavonoids have antioxidative, chondroprotective, and anti-inflammatory activity. It can decrease MMP-3 expression, therefore preventing the degradation of articular cartilage's main component (protein helix) and the irreversible damage in cartilage matrix structure.<sup>27</sup>

Previous study found that honey could to reduce Tumor Necrosis Factor- $\alpha$  (TNF- $\alpha$ ) level in serum compared to its observed level in the untreated group of the osteoarthritic rat model. Honey was also responsible for the inhibition of Vascular Endothelial Growth Factor (VEGF) pathways related to the destruction of joints and pain in osteoarthritis. However, Jimoh-Abdulghaffaar and Owoyele found that honey in lower doses was more effective compared to higher doses to reverse disease development and exhibit anti-inflammatory potential for MIA-induced osteoarthritis model in Wistar rats based on TNF- $\alpha$ , prostaglandin E2 (PG E2), cartilage oligomeric matrix protein (COMP) and vascular endothelial growth factor (VEGF) level.<sup>28</sup>



**Figure 1.** Mean *Matrix Metalloproteinase-3* (MMP-3) level (pg/ml) in patello-femoral articular cartilage.

TG1: TH 25%+PSE 1%, TG2: TH 25%+PSE 5%, TG3: TH 25%+PSE%, TG4: TH 50%+PSE 1%, TG5: TH 50%+PSE 5%, TG6: TH 50%+PSE 10%, PC: positive control, NC: negative control.

\* $p < 0,05$  compared with negative control

The combination of herbal compounds is regarded as superior compared to individual herbal regiments in terms of potency and safety. It is based on the potential synergistic effects and the ability to overcome any potential toxicity.<sup>29</sup> Synergistic works of compound related to the better oral bioavailability and slower elimination rate (prolonged time for maximal concentration), which in turn exert more significant therapeutic effects, with the contribution of hepatic and intestinal metabolism.<sup>30</sup> Previous in vitro research on knee osteoarthritic cells showed that combination of curcuminoids extracts, green tea extract, and hydrolyzed collagen had the potential effect to down-regulate pro-inflammatory gene expression and protein production, i.e., IL (interleukin)-1 $\beta$  stimulated chemokine ligand 6, bone morphogenetic protein-2, matrix metalloproteinase-13 (MMP-13), and stanniocalcin1.<sup>31</sup> Animal trial on Wistar rats model of collagenase-induced osteoarthritis shows that *Sida cordifolia* L. and *Zingiber officinale* mixture down-regulate matrix metalloproteinase-3 and 9 (MMP-3 and MMP-9) as well as up-regulating tissue inhibitors of metalloproteinases (TIMPs) that is related to classical pathogenesis of osteoarthritis.<sup>32</sup> Result from the previous study also demonstrated that *Rosa* spp enhanced the antioxidative activity of honey by increasing free radical-scavenging capacity, decreasing the lipid peroxidation process, and contributing to the increase of phenolic content and vitamin C.<sup>33</sup> Better anti-inflammatory profile related to the combined herbs related to several pathways, including bioavailability enhancement, synergistic antioxidant capacity, preservation of gut integrity and gut microbiome targeting, and broader target of action (cells, signaling pathways, and inflammatory markers).<sup>34</sup>

## CONCLUSION

Syrup made of peanut shells extract and *Apis dorsata* honey significantly decrease inflammatory marker (MMP-3) level in MIA-induced osteoarthritic rats in a dose-dependent manner. Therefore, it could be used as a novel osteoarthritis alternative treatment choice due to its anti-inflammatory and chondroprotective activity. However, further study using an X-ray of the knee or histopathological examination is needed to support this statement. Evaluation of daily activity and pain scoring may also be used to evaluate the clinical significance

further. Our limitation also includes the absence of the control group, composed of only Tualang Honey or peanut shell extract. Future research can also include a single material examination to evaluate the consistency of the dose-dependent manner of therapeutic effects.

## ACKNOWLEDGEMENTS

We would like to thank the Ministry of Research, Technology, and Higher Education of the Republic of Indonesia for funding this study. We also want to show our gratitude to Mrs. Maisa Pusrita and Mrs. Laila Wardani (Sriwijaya University) as laboratory operators for helping us to conduct this study.

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