The Potential of Forest Honeybee on Decreasing Malondialdehyde Levels in Adult with Mild Acne Vulgaris

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Abstract: Acne vulgaris is a common skin disorder, and it mainly affects adolescents. It affects more than 85% of the population, age 12-24 years, and it is described as chronic inflammation of the pilosebaceous gland with the distribution of the lesions mainly on the face, back, chest, and upper arms. Reactive Oxygen Species (ROS) and oxidative stress play a role in the development of inflammatory, acne lesions. To protect against ROS, the human body has an organized antioxidant system, which works synergistically. Antioxidants protect cells against oxidative damage and can prevent the production of oxidative products. An imbalance between oxidants and antioxidants, where the production of ROS exceeds antioxidant capacity, has the potential to cause damage, which is called oxidative stress. One of the biomarkers of oxidative stress in cells is lipid peroxidation and the end product known as malondialdehyde (MDA). The antioxidants in honey can reduce lesions in acne vulgaris. The experiment results showed the honey decreased malondialdehyde level and reduced lesion in acne vulgaris.

1 INTRODUCTION

Indonesia has a surface area of 1.91 Mkm² and with its more than 18,000 islands and ~113 Mha (2010) of forest area, the country harbors flora and fauna rich in diversity (Abood, Lee, Burivalova, Garcia-Ulloa, & Koh, 2015; Cribb & Ford, 2009; Hansen et al., 2013; United Nations, 2018). The remarkable diversity is also reflected by the number of bee species. Thus, 8 out of 9 species of the genus Apis, with Apis laboriosa as the only absent one, as well as more than 40 stingless bee species were observed in Indonesia (Gupta, Reybroeck, van Veen, & Gupta, 2014).

It has been estimated that 66% of the world’s crop species are pollinated by bees, including honey bees, bumble bees and solitary bees (Kremen, Williams, & Thorp, 2002; Partap, 2011). Beekeeping not only positively contributes to income gain, it also plays a role in increased food security, but beekeeping activity and its potential receives only subordinate attention within the Indonesian government and population. According to scientists from the Universitas Padjadjaran (UNPAD, Bandung, Indonesia), bee businesses are mostly considered as a part time farming activity and not only parts of the local community, but people from every social class are not aware of the bees’ benefits (Chantawannakul, Williams, & Neumann, 2018). As found in a survey by UNPAD and CV. Primary Indonesia (Labtek Indie), among 80 citizens 57.5% had certain prejudices against bees. Those range from insufficient profitability, to fear of bee stings, to a lack of knowledge on the importance of bees as pollinators. Furthermore, there are field owners fearing bees have a negative impact on their crops’ productivity. They do not want bees or beehives near their property and in a consequence some of them are willing to burn those colonies, if verbal warnings were ignored by the beekeepers. Indonesia is the 4th most populous country worldwide with a population that reached 264 million in 2018 (FAOSTAT, 2018). The population growth is accompanied by a significant stress for the Indonesian ecosystem and a continuous increase of used land area, triggered by rising demands of natural resources like timber and food (Abood et al., 2015). To antagonize the existing trend, beekeeping can be used to sensibilize the population towards the...
importance of forest conservation and non-timber materials.

So far, local beekeepers use mostly native honey bee species like Apis cerana or Meliponini colonies for managed beekeeping (Figure 2) (Schouten, Lloyd, & Lloyd, 2019), but it is also common to practice the art of honey hunting from wild living, so far not-manageable, Apis dorsata colonies (Crane, Van Luyen, Mulder, & Ta, 1993; Gupta et al., 2014). Besides honey hunting and beekeeping, Bradbear and FAO (2009) defined a third type of apicultural activity: “Bee maintaining”; an intermediary stage of beekeeping, where humans safeguard wild living colonies. The colonies are not kept in hives, but honey collectors often provide artificial nesting places, for example traditional tikung, tingku or also called sunggau (wooden honey boards or tree trunks) (Hadisoesilo, 2002). A similar method to maintain A. dorsata colonies, observed in Indonesia and elsewhere in South-East Asia, is the use of special rafters (Bradbear & FAO, 2009; Crane et al., 1993). Tikungs are trapezium shaped boards often made from banyan (Ficus benghalensis) wood, which are placed between tree branches to attract feral A. dorsata colonies. If the tikung is occupied, the bees build their nest on it while they forage on the same and on neighboring trees in flower. Harvesting takes place during the rainy season and honey collectors cut only the top of the honey containing part of the comb to protect the brood and to maintain the colony. To obtain the honey and separate it from beeswax, it is not common to squeeze, but gently let the honey flow through filter fabric (WWF, 2010) (Figure 1). Once a tikung is occupied by a swarm, it is believed, that the same colony remigrates to it every year (Paar, Oldroyd, Huettinger, & Kastberger, 2004). This method may be a good alternative to the less secure and more common practice of honey hunting and is also used in other Asian countries (de Jong, 2000; Mahindre, 2000).

Plants also have been used for healing in many cultures for centuries. Generally, plants act to stimulate and supplement the body’s healing process (Nwanko C.M, Ezekoye C.C, and Igboke S.O, 2014). The problem: limited use in modern medicine due to lack of scientific evidence supporting it (Tahereh Eteraf Oskouei and Moslem Najafi 2012).

Honey is a viscous liquid produced by bees from nectars and flowers; the active components of honey such as glucose, fructose, flavonoid, polyphenols, and organic acid play an important role in its quality (Serene Hilliary, 2017; Visweswara Rao Pasupuleti, 2017). Traditional medicine around the world has described honey as efficacious in the treatment of various skin disorders. Some have assumed that it has healing properties in regards to acne vulgaris (Pauline Mcloone, 2016; Khoiroh Umah and Oriza Herdanti, 2017).

Honey is highly rich in bioactive compounds such as poliphenols and vitamins. Phenolic compounds are bioactive compounds; phenols are defined as organic compounds with an aromatic ring which is chemically bonded to one or additional hydrogenated substituents in the presence of corresponding functional derivatives. In honey, phenolic compounds are commonly present as flavonoids. Antioxidative in honey produces anti-inflammatory effects which may reduce localized inflammation that develops with acne. Honey is a complementary therapy dating back to hippocrates.

Raw unprocessed wild forest honey sustainably harvested by the indigenous communities of Sumbawa, Flores, Kalimantan, Sulawesi and Sumatra. Each island has a distinct flavour profile ranging from delicate and floral to rich and intense, reflecting the diversity of the regional ecosystems.

100% raw and unheated, as a result our honey retains all of nature's goodness: the pollen, enzymes, vitamins, amino acids, antioxidants, propolis, minerals and its natural flavour.

The forest honey comes from the 'Apis Dorsata', the giant rock bee. It is the biggest bee in the world at about 2.5 cm long. This wild bee cannot be domesticated like other honeybees.

Sustainable harvesting of wild forest honey supports the indigenous communities and helps protect the rainforests of Indonesia.

Prophet Muhammad used honey for one week to treat acne, honey was applied in the area of acne, one or two hours before going to sleep and left until morning, to reduces acne vulgaris (Visweswara Rao Pasupuleti, 2017; Darul Hadrahah, 2014; Alex Seprini, 2015). Honey have been used as an anti-inflammatory agent for centuries, used honey to treat patients (Gunawan, 2017).

In ancient Greece, Pedanius Dioscorides, a doctor and pharmacologist, used honey to treat patients (Gunawan, 2017). The four etiologies are hyperproliferation of follicular epidermis, excess sebum production, Propionbacterium acnes bacteria, and inflammatory reactions.

The term of “acne” is derived from the greek word “acme” which is used to describe the peak and vulgaris which means “common” (Zohra FT, 2017; Ginard I Henry, 2018). According to a study from
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Inflammation is a chronic inflammatory disorder of the pilosebaceous gland which manifests clinically in the form of blackheads, papules, pustules and cysts (Darren D lyyn, 2016; Irma Bernadette, S. Sitoehang and Sjarif M. Wastaatmaja, 2017; Amit Batra, 2014). Honey have been used as an anti-inflammatory agent for centuries.

Acne is often to be correlated with oxidative stress mechanism in adults. Oxidative stress is an imbalance between oxidants (free radicals) and antioxidants. The damage that occurs due to an imbalance between production and issued ROS (Reactive Oxygen Species) on the skin. ROS in the skin comes from normal cell metabolism; for example: mitochondrial respiration and enzymatic activity. Additionally, ROS derived from exogenous factors is produced by various environmental factors, such as UV light or the presence of a chronic inflammatory process on the skin. To regulate the level of ROS, the skin has an antioxidant defense system, which goal is maintaining homeostasis. (Amand wong, 2016) One of the biomarkers of oxidative stress in the cells is lipid peroxidation and the end product known as malondialdehyde (MDA).

The physiological process of the emergence of free radicals in the body is closely related with prooxidant; it is balanced with an endogenous defense mechanism by producing substances that have anti-oxidative properties (Euis Reni Yulianti, 2017). Honey is known as a natural antioxidant; lately many studies have focused on the composition of honey and its biological properties as an antioxidant, anti-inflammatory and antimicrobial, which are thought to be effective in treating skin problems (Lee Suan Chua, 2013).

Oxidative stress can cause disturbances in biological functions such as ion homeostasis, enzyme activity, membrane integration, cell function, even cell damage or death (Euis Reni Yulianti, 2017). Several studies have been carried out to determine the trigger of oxidative stress in the pathogenesis of acne vulgaris. The lipid oxides formed can activate proinflammatory cytokine production and activation of Peroxisome proliferator-activated receptor (PPAR). PPAR is a nuclear transcription factor that helps in lipids and inflammatory reactions. PPAR-α is often involved with β-oxidation of fatty acids and lipid catabolism, whereas PPAR-γ is associated with the process of lipidogenesis. PPAR-vasi activation can also induce the expression of cyclooxygenase-2 (COX-2) and prostaglandin E2 (PGE2), causing further inflammation in the pathogenesis of Acne vulgaris (Sylvia Anggraeni, 2017). Recent research reports the possible role of reactive oxygen species (ROS) in particular fat peroxidation in the onset of inflammation. Malondialdehyde (MDA) is the end result of lipid peroxidation which is commonly use as a marker of lipid peroxidation. This study was conducted to examine the relationship between serum MDA levels and the severity of acne in male patients.

According to a study by Dr. Sardjito, a total of 60 patients with acne visited the Skin Polyclinic; RSUP Dr. Sardjito who fulfilled the inclusion and exclusion criteria was involved in the study. From their research, patients were grouped into three groups, namely patients with mild, moderate and severe acne vulgaris. Blood samples of all the patients were taken and the serum MDA levels were determined with a spectrophotometer. The mean serum MDA level in patients with acne vulgaris increased. The mean serum MDA levels of severe acne group (1.85 ± 0.368 μmol/L) were significantly higher than that moderate acne group (0.79 ± 0.159 μmol/L) (p = 0.000) and that mild acne group (0.52 ± 0.239 μmol/L) (p = 0.000) (Aprilina Dwi Sulistyowati, 2014).

The ability of honey to prevent oxidative damage may be caused by its phenolic antioxidant content which may have triggered the modulation of antioxidant enzyme activity. (Zulaikha Sahhugi, et al, 2014). The antioxidative and anti-inflammatory properties of honey have been recognized by various studies. It is related to the content of polyphenols contained in honey. All types of honey contain a mixture of sugar, protein, vitamins, minerals, polyphenols, and antioxidative mostly from polyphenols. In one study (Serene Hilary, et al, 2017), honey had an antioxidative effect which was characterized by a decrease in MDA levels in the blood.

Honey releases hydrogen peroxide, which is an antibiotic that can also remove bacteria and clear acne. The anti-inflammatory properties of honey reduce the redness of acne. Its acidic property stops bacteria from growing (Elin Julianti, 2017). The clinical severity of acne in this study was assessed using a grading system, participants were grouped into 2 subgroups: mild and moderate acne vulgaris. The aim of the study was to investigate the use of honey masks in decreasing malondialdehyde level and reducing lesions in acne vulgaris.

The aim of the study was to investigate the use of honey masks in decreasing malondialdehyde level and reducing lesions in acne vulgaris.
2 RESEARCH METHODOLOGY

2.1 Materials and Instruments

Materials used were honey acquired from the forest. NaOH, Mayer Reagen, Gelatin Solution 1%, Aquadest (distilled water), chloroform, anhydride acid, and sulfurate acid, Trichloroacetic acid (TCA) 40%, Hypochlorite acid (HCl), Na-thiosulfate (NaThio) 1 N, 3cc of Blood Samples. Instruments used were micro pipets, cuvette rack, gloves, tourniket, spuit, centrifuge 1 unit, Sample rack 1 unit, microtube, cuvette, waterbath, UV-Spectrophotometer, test tubes, and shaving brush.

Honey used in this study was purchased from local bee keepers in North Sumatera, Indonesia. Honey, which had been tested for phytochemical, was packaged and given to the participants. The honey was applied once per day for seven days as a mask; 7 packages were given per participant and each package contained 10cc.

2.2 Phytochemical Analysis

Before applying honey, a phytochemical analysis was carried out to determine its antioxidant content. In this study, qualitative test for phytochemical such as flavonoids, alkaloids, Tannins, Saponin, and terpenoids content of honey samples were carried out.

2.2.1 Detection of Flavonoids

Alkaline Reagent Test: A few drops of diluted NaOH were added to the extracts. An intense yellow colour was produced and became colorless after of a few drops of diluted acid were added; this indicated the presence of flavonoids (Asokan S and Jayanthi, 2017).

2.2.2 Detection of Alkaloids

Mayer’s Test: 50 cc of solvent free extracts were stirred with a few ml of diluted HCL and filtered; then a few drops of Mayer’s reagent were added by dripping on the side of the test tube. The white or creamy precipitate indicated a positive result (Asokan S and Jayanthi, 2017).

2.2.3 Detection of Tannins

Gelatin Test: A 1% gelatin solution containing sodium chloride was added to the extract.

Formation of a white precipitate indicated the presence of tannins (Prashant Tiwari, 2011).

2.2.4 Detection of Saponins

Distilled water (2 ml) was added to each extract and shaken in a graduated cylinder for 15 mins lengthwise. Formation of 1 cm foam indicated the presence of saponin (Asokan S and Jayanthi, 2017).

2.2.5 Detection of Terpenoids

Chloroform (2 ml) and concentrated sulphuric acid was added carefully to 0.5 ml of extract. Formation of red-brown colors at the surface indicated the presence of terpenoids (Asokan S and Jayanthi, 2017).

2.3 Participants

In this study, the participants were grouped into mild acne vulgaris and assessed using a grading system. Participant’s criteria should be young adults with acne vulgaris aged 18-22 years, and have not received acne therapy for the previous two weeks either topical therapy or systemic therapy, and a willingness to be included in the study. Participants were grouped based on levels of severity according to the IGA scoring system: mild (IGA 2); informed consent sheets were given to all the participants.

Table 1: Investigator’s Global Assessment (IGA) Scale (Linda Stein Gold, 2017)

<table>
<thead>
<tr>
<th>Levels of Severity</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clear (IGA 0)</td>
<td>Clear Skin with no inflammatory or non inflammatory lesions</td>
</tr>
<tr>
<td>Almost Clear (IGA 1)</td>
<td>A few Scattered comedones and a few small papules</td>
</tr>
<tr>
<td>Mild (IGA 2)</td>
<td>Easily recognizable. Less than half the face is involved, some comedones and some papules and pustules</td>
</tr>
<tr>
<td>Moderate (IGA 3)</td>
<td>More than Half of the face is involved, many comedones, papules and pustules</td>
</tr>
<tr>
<td>Severe (IGA 4)</td>
<td>Entire face is involved, covered with comedones, numerous papules and pustules, few nodules may or may not be present</td>
</tr>
</tbody>
</table>

Lesions were calculated before and after applying honey as a mask for 7 days using the criteria from Fakultas KedokteranUniversitas Indonesia the method of calculating lesions showed in table 2.
Table 2: Criteria for severity of Lesions

<table>
<thead>
<tr>
<th>Acne Degree</th>
<th>Blackheads</th>
<th>Inflammatory Lesions</th>
<th>Total Lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>&lt;20</td>
<td>&lt;15</td>
<td>&lt;30</td>
</tr>
<tr>
<td>Moderate</td>
<td>20-100</td>
<td>15-30</td>
<td>30-125</td>
</tr>
</tbody>
</table>


Procedure:
1. Student whom suffered acne vulgaris at Fakultas Kedokteran Universitas Prima Indonesia were chosen. Student that gave their approval was accepted into the study.
2. Based on the physical examination, the research subjects were grouped according to levels of acne vulgaris mild and moderate.
3. Examination of blood before honey is applied.
4. Participants were given 7 packages of honey as a mask that was used for 7 days at a dose of 10 ml per pack. Participants were required to wash their faces before using honey when they wanted to sleep. After the mask was dry, the participants rinsed their faces with water.
5. After using the masks for 1 week, blood samples of participants were extracted at 3cc/participants.
6. Examination of MDA levels.
7. Analysis of data.

Measurement of Malondialdehyde Level has been conducted as the following:
1. Blood samples were inserted into a rack blood samples were inserted into the Centrifuge machine and centrifuged at 2500 rpm for 8 minutes to produce serum.
2. Serum was taken out using a micropipette and inserted into microtube.
3. 0.5 cc of serum added with: (1) TCA 40% 2.5 µl, (2) 200 µl HCl 1 N, (3) 0.5 cc aquabides, and (4) NaThio 1% 100 µl.
4. It was then heated at 100 °C for 25 minutes using a water bath machine.
5. Afterwards it was centrifuged at 3000 rpm for 5 minutes and then the formed supernatant was taken out.
6. The supernatant was put in a vacuum tube and aquabides (3 cc) was added.
7. Then the supernatant was inserted into the cuvette, then the cuvette was inserted into the UV-Spectrophotometer machine. Then amount of absorption at a wavelength of λ 532 was measured.

3 RESULTS AND DISCUSSION

Phytochemical screenings of honey samples have showed the presence of flavonoid, alkaloid, saponin, and terpenoids. The result of phytochemical screening of honey can be seen in the table 3 below:

Table 3: Result of Phytochemical Screening of Honey Samples

<table>
<thead>
<tr>
<th>Phytochemical Content</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoid</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloid</td>
<td>+</td>
</tr>
<tr>
<td>Tannin</td>
<td>-</td>
</tr>
<tr>
<td>Saponin</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoid</td>
<td>+</td>
</tr>
</tbody>
</table>

The aim of this phytochemical screening of honey samples was to investigate its bioactive compounds such as flavonoid, alkaloid, tannin, saponin, and terpenoid. Honey contains vitamins which may serve as sources of polyphenol and dietary antioxidants. Honey mainly consists of sugars and water but also contains several vitamins, especially B complex and vitamin C, and minerals. It possesses antibacterial, anti-inflammatory, and antioxidative properties that may be beneficial for combating multi-drug resistant bacteria; additionally, it may also be useful for preventing chronic inflammatory processes, such as acne vulgaris. (M. Assaduzaman, 2015; Halah Musthafa Ya'qub, 2013).

Table 4: The T-test of MDA levels in Mild Acne Vulgaris

<table>
<thead>
<tr>
<th>Degree of Acne Vulgaris</th>
<th>Mean</th>
<th>T-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before Application of Honey and MDA Measurement (N= 35)</td>
<td>Mild</td>
<td>0.909 (P= 0.000)</td>
</tr>
<tr>
<td>After Application of Honey and MDA Measurement (N= 35)</td>
<td>Mild</td>
<td>0.808 (P= 0.000)</td>
</tr>
</tbody>
</table>

Table 4 above showed a decreased MDA level after application of the honey. In this study, an Independent T Tests was carried out before and after MDA measurement in mild acne vulgaris. A mean score of 0.909 in mild acne vulgaris was calculated.
After the application of honey for 7 days, a mean score of 0.808 in mild acne vulgaris was calculated. This shows a statistically significant decrease in MDA level.

Honey was shown to have anti inflammatory activities which reduced acne vulgaris lesions. There was a visible improvement after the use of honey masks. Similar results were reported by Khoiroh Umah and Oriza Herdanti. Their studies used statistical tests gained from Paired Sample Tests which resulted in the rejection of the null hypothesis with a value of $\alpha = 0.000$. This meant that the students that had undergone treatment using the honey had statistically significant differences in the amount of acne vulgaris compared to before the application of the honey. The study was conducted by PSIK students from Gresik University (Khoiroh Umah and Oriza Herdanti, 2017).

In this study, an Wilcoxon Signed Ranks Test was carried out before and after the application of honey masks to mild acne vulgaris, it can be seen in table 5 below:

<table>
<thead>
<tr>
<th>Degree of Acne</th>
<th>Wilcoxon Signed Ranks Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before Application of Honey in Mild Acne Vulgaris (N=35)</td>
<td>$t = -5.173$ (p = 0.019)</td>
</tr>
</tbody>
</table>

The normality test (Shapiro-Wilk) shows a p value of less than 0.05; therefore, the null hypothesis, that the data distribution is normal, is rejected. Dependent T test is a test to determine if there is a statistically significant difference before and after the application of honey on the amount of acne. Since only the data of people with moderate acne (Before & After) is normally distributed, the dependent t-test is used. The result: a p value of less than 0.01 for people with a moderate amount of acne. This meant that the null hypothesis, there was no difference between before and after the application of honey, was rejected. It can be concluded that there was a statistically significant difference between before and after the application of honey (due to the p value) and all of the people (with mild acne) experienced a decrease in the amount of acne (due to all the samples being in the negative rank).

In this study, Dependent T Test was carried out before and after the application of honey masks to moderate acne vulgaris, it can be seen in table 6 below:

<table>
<thead>
<tr>
<th>Acne Vulgaris Degree</th>
<th>Dependent T test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before Application of Honey in Moderate Acne Vulgaris (N=35)</td>
<td>(p = 0.040)</td>
</tr>
<tr>
<td>After Application of Honey in Mild Acne Vulgaris (N=35)</td>
<td>(p = 0.000)</td>
</tr>
</tbody>
</table>

Table 6, normality test (Shapiro-Wilk) shows a p value of more than 0.05; therefore, the null hypothesis, that the data distribution is normal, is accepted. Dependent T test is a test to determine if there is a statistically significant difference before and after the application of honey on the amount of acne. Since only the data of people with moderate acne (Before & After) is normally distributed, the dependent t-test is used. The result: a p value of less than 0.01 for people with a moderate amount of acne. This meant that the null hypothesis, there was no difference between before and after the application of honey, was rejected. It can be concluded that there was a statistically significant difference between before and after the application of honey (due to the p value) and all of the people (with moderate acne) experienced a decrease in the amount of acne.

Application of honey as face masks in mild and moderate acne vulgaris can be seen in figure 1 and figure 2 respectively.
from Paired Sample Tests which resulted in the rejection of the null hypothesis with a value of $\alpha = 0.000$. This meant that the students that had undergone treatment using the honey had statistically significant differences in the amount of acne vulgaris compared to before the application of the honey. The study was conducted by PSIK students from Gresik University (Khoiroh Umah and Oriza Herdanti, 2017).

4 CONCLUSIONS

The MDA level decreased in acne vulgaris patients after the application of honey. Honey releases hydrogen peroxide, which is an antibiotic which can remove bacteria and clear acne. The anti-inflammatory properties of honey reduce the redness of acne. Honey can decrease the MDA level and reduce the lesion of acne vulgaris.

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