

**Effects of Apis Mellifera, Apis Cerana, and Trigona SP on 20 M Sprint Performance****Kusnaedi^{1*}, Mukti Priastomo², I Ketut Adyana³, Sukrasno³**¹Magister of Sports Science, School of Pharmacy, Institut Teknologi Bandung, Indonesia²Faculty of Pharmacy, Mulawarman University, Indonesia³Magister of Pharmacy, School of Pharmacy, Institut Teknologi Bandung, Indonesia**Article Info***Article History :**Received August 2022**Revised August 2022**Accepted August 2022**Available online September 2022**Keywords : honey, human, physical activity***Abstract**

This study aimed to determine the effect of three types of honey from three different honey bee species on increasing stamina and anti-fatigue effect by examining their effects on the biochemical process in the anaerobic pathway. Tests were carried out on humans who had met the specified criteria. The method used in this study was an experimental method involving 30 healthy students. The inclusion requirements consisted of males aged 18-20 years, weight 50-70 kilograms, height 160-170 cm, normal momentary blood sugar levels <140 mg/dL, VO₂max 35-40 ml/kg/min, normal blood pressure, and not having serious diseases such as diabetes, asthma, kidney failure, hepatitis, as well as other serious diseases that hinder physical activity. Volunteers had also signed informed consent stating their willingness to participate in the research to completion. The results of this study showed that there was a change between pre and post in each groups, indicating that each honey content had a significant impact on improving the participant performance. In addition, our study found changes in the three types of honey (Apis Mellifera, Apis Cerana, Trigona SP) in the participant running performance improvement. However, there was no significant difference between the three types of honey.

INTRODUCTION

Honey bees are insects of the Apidae family that gather and transform plant nectar or secretions into honey through foraging (Dümen et al., 2012; Ilija et al., 2021; Miguel et al., 2017; Samarghandian et al., 2017; Wong, 2020). The Apidae family is generally divided into two genera: the Apis or stinging honey bees and Trigona or stingless honey bees (Jose Vazhacharickal, 2021; Miguel et al., 2017; Samarghandian et al., 2017). The number of species of honey bees affects the quantity of honey produced. The color, flavor, and aroma of different kinds of honey can be used to distinguish them physically. The chemical components of honey can also be used to determine the differences between the types of honey (Ali et al., 2021; Jose Vazhacharickal, 2021).

In general, the main ingredients of honey are fructose and glucose. However, in addition to these, honey also contains vitamins, proteins, and minerals, which impact its quality. Occasionally it also contains flavonoids, alkaloids, and other chemical compounds (Wong, 2020; Miguel et al., 2017; Dümen et al., 2012). Honey has a wide range of chemical components, which enables several advantages (Moniruzzaman et al., 2013). (Moniruzzaman, 2013). One of honey's features is typically thought to increase stamina. Further, it is believed that the main factor contributing to increased stamina in activity is the presence of high fructose and glucose consistency. (Ali et al., 2021; Priastomo & Adnyana, 2016; Safitri et al., 2020; Wong, 2020).

One could equate increased energy with increased stamina. Energy consumption necessitates intricate biochemical procedures. Adenosine triphosphate serves as the source of the necessary energy (ATP). This energy in the form of ATP will be hydrolyzed into adenosine diphosphate (ADP). Fatigue results from the limited supply of energy in the body (Pharaoh et al., 2021). The fatigue parameter is due to the formation of lactic acid. Without glycolysis and an accumulation of unmetabolized lactic acid, which can cause acidosis and cause pain, the production of lactic acid as a metabolite is achieved through anaerobic metabolism pathways using glucose and glycogen (Brooks, 2018; Proia et al., 2016) (Proira, Brooks).

Previous research has demonstrated how nutritional supplements like green tea extract, curcumin, and

cinnamon increase stamina and anti-fatigue effects in individuals and athletes (da Silva et al., 2018; Yoon et al., 2020). However, there have not been any studies on interventions using honey on individuals or athletes that affect increasing stamina and anti-fatigue effects. Therefore, this study aimed to investigate the effects of three types of honey from three different honey bee species on enhancing stamina and reducing fatigue by examining their effects on anaerobic pathway biochemical processes. Individuals who have fulfilled the requirements participated in the tests.

METHODS

The research process started with an observation, followed by the selection of honey sampling sites, identification of the species of honey bees, and evaluation of the honey's production quality. Next, *Apis mellifera*, *Apis cerana*, and *Trigona* sp. honey were collected and prepared for testing. Anti-fatigue tests were conducted first on experimental animals and then on human participants.

Male mice that had met the inclusion criteria for age and body weight were used as experimental animal models for the anti-fatigue test. The Weight-loaded Forced Swimming Test (WFST) procedure was employed for the test. The variables measured included swimming duration, blood lactate and glucose concentrations, and liver and muscle glycogen levels (*M. gastrocnemius*).

Test results on experimental animals were applied to human participants using the Running Based Anaerobic Sprint Test (RAST) method. The measurement parameters were running time, blood glucose levels, blood lactic acid levels, speed, power, and VO₂ max.

This study used an experimental research design with a single group for both the pretest and posttest, with no additional groups or controls. If they choose to take part in the entire series of research, all research participants must fill out informed consent forms as a condition of participation. The research protocol conducted had been approved by the ethics committee of the Ministry of Health, POLTEKES Bandung.

Participants

Thirty healthy college students took part in this study, with inclusion criteria consisting of males aged between 18-20 years, body weight 50-70 kilograms, height 160-170 cm, and normal blood sugar levels on short-term intake of < 140 mg/dL, VO₂ max 35-40 ml/kg/min, normal blood pressure, and does not have serious diseases such as diabetes, asthma, kidney failure, and hepatitis, as well as other serious diseases that hinder physical activity. Volunteers have also completed an informed consent form indicating their willingness to participate in the research.

Material

Trigona sp honey, Apis cerana honey, Apis mellifera honey, filter paper, aluminum foil, lead and copper, aqueous ammonia, chloroform, hydrochloric acid, Dragendorff reagent, Mayer's reagent, magnesium powder, amyl alcohol, iron (III) chloride, gelatin, ether, Lieberman-Burchard reagent, aluminum foil, and energy drink preparations containing caffeine, aqua dest, rough tissue, Eppendorf, 2.5 cc syringe, plaster, cotton swab, glucose strip (Easy Touch®), hemoglobin strip (Easy Touch®), lactate (liquid) reagent set (Pointe Scientific Catalog L7596.50), alcohol, Anthrone Acs reagent 97% (Sigma Aldrich®), aluminum foil, concentrated sulfuric acid, and KOH.

The equipment used consists of the water bath, cuvette, evaporating dish, atomic absorption spectrophotometer, furnace, laminar air flow, test tube, volume pipette, dropper, test tube rack, tube clamp, Centrifugation tube, semi-automated Clinical Chemistry Analyser, Vital Scientific (microlab 300®), General Purpose UV/Vis Spectrophotometer DU 720 (Beckman Counter®), Micropipet (Socorex®), Glucose test (Easy Touch®), stopwatch, digital gram scale, digital kilogram scale (Kris®), electron microscope, Erlenmeyer flask, test tube rack, measuring cup, measuring flask, beaker, test tube, dropper pipette, volume pipette, mouse pool, scissors, restrainer, porcelain crucible, hot plate, oral probe, digital sphygmomanometer, and various supporting glass.

Procedure

Honey Characteristic Test

Honey requirements based on the Indonesian National Standard include the activity of the enzyme dia-

stase, hydroxymethylfurfural (HMF), acidity, ash content, water content, glucose, sucrose, reducing sugar, metal contamination, and insoluble solids.

Diastase enzyme determination

To prepare the sample, 5 grams of honey were put into a 20 ml beaker, 10 ml – 15 ml of water, and 2.5 ml of acetate buffer solution. In a cold state, the solution was stirred until the honey sample was completely dissolved. Next, this sample solution was transferred into a 25 ml volumetric flask containing 1.5 ml of NaCl solution, adjusted to the tera mark with water (the solution must be solidified before adding NaCl solution).

To determine absorbance, 10 ml of honey sample solution was pipetted into a 50 ml test tube and placed in a 400 ± 0.20°C water bath along with an Erlenmeyer flask containing starch solution. Turning on the stopwatch, 5 ml of the starch solution was pipetted into the sample solution after 15 minutes. Next, one milliliter of the sample mixture was pipetted into ten milliliters of the iodine solution at five-minute intervals. The absorbance value was calculated at a wavelength of 660 nm after mixing and then diluted to volume. The reaction time was measured from the point at which the starch and honey were combined to the point at which the liquid was added to the iodine (put a 1 ml pipette in the test tube to be reused when the liquid was taken back), and kept taking the solution every predetermined amount of time until the result was A 0.235.

Iodine and starch solution combined will result in blue color. This is because diastase enzymes will convert starch into sugar, and the diastase enzyme will cause the blue color in the starch solution to vanish. The higher the enzyme activity, the faster the loss of the blue color from the starch.

The determination of Hydroxymethylfurfural (HMF)

A carefully weighed 5 g of honey (to an accuracy of 1 mg) in a small glass was put into a 50 mL volumetric flask and rinsed with water until the solution volume was 25 mL. 0.50 mL of Carrez I solution was added, then shaken before adding 0.50 mL of Carrez II solution, shaken once more, and diluted with water up to the line mark. Added a drop of alcohol to remove the foam on the surface. Filtered through filter paper and discarded the first 10 mL of the filter. 5 mL of the sieve was then pipetted into an 18 mL x 150 mL test tube. 5 mL of water was pipetted into one tube (example), and 5

mL of 0.20% sodium bisulfite into the other tube (comparison). After completely mixing, the absorbance of the sample was determined against the reference (comparison) in a 1 cm cell at a wavelength of 284 nm and 336 nm. If the absorbance was higher than 0.6 to obtain precise results, the sample solution was diluted with water as required. Likewise, the reference solution was diluted in the same way using 0.1% NaHSO₃ solution; the absorbance value obtained was multiplied by the dilution factor before calculation.

The determination of acidity levels

After carefully weighing 10.0 g, the honey was put into a 250 mL Erlenmeyer flask, dissolved with 75 mL of distilled water, and 4-5 drops of PP indicator. After that, it was titrated with 0.1 N NaOH solution to a fixed end point for 10 seconds. The volume of 0.1 N NaOH used for the titration was recorded. Alternatively, a pH meter can be used, and the sample titrated to a pH of 8.3. Then the acidity of honey was calculated.

The determination of ash content

A total of 3 g of honey was weighed carefully and put into a porcelain crucible that had been ignited. The crucible was slowly heated to a temperature of 500-600°C until the charcoal ran out, cooled, and weighed. The ash content of the sample was the difference between the weight of the porcelain crucible containing ash and the weight of the empty porcelain.

The determination of water content

Three drops of honey were smeared on a hand refractometer. The tool serves to see the results of the reflection of the honey to measure the water content in the material.

Test of Lead (Pb) and Copper (Cu) Levels

A porcelain cup held the carefully measured 5.0 grams of honey. The honey sample was heated on an electric stove for a few hours or until the white smoke turned to charcoal. To obtain ash, samples turned to charcoal were heated vigorously in a furnace at a temperature of 500–600°C. The ash was next soaked in diluted HNO₃. The sample bath was filtered, placed in a measuring flask, and added 50 mL of distilled water. Standard lead (Pb) and copper (Cu) solutions were created for comparison. The concentrations were 100, 10, 8, 6, 4, 2, and 1 parts per million. The results of the soaked samples that had been filtered were measured

using atomic absorption spectroscopy (AAS) instrument and compared with the raw materials of lead (Pb) and copper (Cu) that had been made.

Pharmacological activity test in human participants

In the first stage, participants who met the criteria for inclusion went through preliminary testing. The test was conducted without any treatment. Participants were asked to run on a track as far as 20 meters at maximum speed. The next phase was an anti-fatigue test. The anti-fatigue testing procedure required participants to perform the anaerobic exercise in the form of sprints over a 20-meter distance. With a stopwatch, time was measured.

Data evaluation

All data displayed were presented in the form of mean ± standard deviation (SD). Differences in parameters that have different sizes were analyzed statistically using the ANOVA test (analysis of variance). The difference was declared significant if $p < 0.05$.

RESULT

The findings indicated that all samples in this study were homogeneous because there were no significant differences in the mean (SD) values of age, height, weight, and BMI between groups.

Table 1. Anthropometric data

Variables	Group 1	Group 2	Group 3
Age (years)	19.7 ± (0.70)	19.6 ± (0.72)	19.5 ± (6.70)
Weight (kg)	62.4 ± (8.69)	61.3 ± (6.70)	59.8 ± (5.3)
Height (cm)	164.7 ± (0.04)	161.7 ± (0.09)	162.1 ± (0.05)
BMI (kg/m ²)	22.92 ± (2.11)	23.65 ± (2.91)	22.17 ± (1.70)

According to the study, there is a difference between the pre-and post-test scores in each group, providing evidence that each type of honey significantly improves the participants' performance. The findings, however, indicated no significant difference between the three groups, according to ANOVA. This demonstrates that the advantages of all three types of honey are similar. Tables 2, 3, and 4 show the outcomes.

Table 2. The effects of *Apis mellifera*, *Apis cerana*, and *Trigona* sp on each group

	Group 1	Group 2	Group 3
pre	9.13 ± 0.59	9.25 ± 0.33	9.15 ± 0.28
post	8.62 ± 0.61	8.82 ± 0.46	8.85 ± 0.31
p = value	0.077	0.028	0.038

Table 3. The effects of *Apis mellifera*, *Apis cerana*, *Trigona* sp on each Pretest group based on paired sample test

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Grp 1 – Grp 2	-.12000	.67690	.21406	-.60423	.36423	-.561	9	.589
Grp 1 – Grp 3	-.02000	.63454	.20066	-.47392	.43392	-.100	9	.923
Grp 2 – Grp 3	.10000	.49441	.15635	-.25368	.45368	.640	9	.538

Table 4. The effects of *Apis mellifera*, *Apis cerana*, *Trigona* sp on each Posttest group based on paired sample test

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Grp 1 – Grp 2	-.19510	.70271	.22222	-.69779	.30759	-.878	9	.403
Grp 1 – Grp 3	-.22510	.56448	.17850	-.62890	.17870	-1.261	9	.239
Grp 2 – Grp 3	-.03000	.52292	.16536	-.40407	.34407	-.181	9	.860

DISCUSSION

Our research shows a change in performance between pre and post-treatment in each group. This supports several theories regarding the benefits of consuming honey (Ali et al., 2021; Jose Vazhacharickal, 2021; Priastomo & Adnyana, 2016; Safitri et al., 2020; Samarghandian et al., 2017). According to the research, using honey as an intervention significantly improved the research subjects' performance. Furthermore, our research supports earlier findings that administering honey in the proper dose can enhance swimming ability and reduce fatigue (Priastomo & Adnyana, 2016). Additionally, other studies that used honey as a sports gel arrived at the same conclusion, showing that consuming a honey-contained drink in the form of a sports gel before exercise provides advantages in the form of improving running performance (Aly et al., 2019).

Further, niacin, pantothenic acid, boitin, folic acid,

water, sugars (fructose, glucose, maltose, and sucrose), minerals (ca, na, p, fe, mg, and n), vitamins (B1, B2, B5, B6, C, and K), and enzymes are some of the ingredients that are found in honey (Jose Vazhacharickal, 2021; Samarghandian et al., 2017; Wong, 2020). Good honey is honey that passes the following quality standards in lab tests: maximum water content of 22%, minimum reducing sugar of 60%, maximum sucrose of 10%, maximum acidity of 40mL NaOH/kh, and minimum diastase enzyme activity of 3 DN (Miguel et al., 2017; Samarghandian et al., 2017).

Furthermore, we try to explain that some of the components that make up honey are air, sugars (fructose, glucose, maltose, sucrose), minerals (Ca, Na, P, Fe, Mg, Mn), vitamins (B1, B2, B5, B6, C, K) niacin, pantothenic acid, boitin, folic acid and enzymes (Jose Vazhacharickal, 2021; Samarghandian et al., 2017; Wong, 2020). Good honey is honey that meets the re-

quirements through laboratory tests, namely: a maximum water content of 22%, a minimum of 60% reducing sugar, a maximum of 10% sucrose, a maximum of 40mL NaOH/kh, a diastase enzyme activity of at least 3 DN (Miguel et al., 2017; Samarghandian et al., 2017). Some of these theories presuppose that after consuming honey, the blood plasma content rises to fight oxidation at higher levels. Additionally, honey contains phenolics that have been proven to significantly increase the body's ability to withstand stress (Aly et al., 2019; Kunugi & Ali, 2019; Miguel et al., 2017; Proia et al., 2016; Bangroo et al., 2005; Khatri et al., 2005). The phenolic has a positive effect on the changes in performance of each participant.

As a result of this research, we found that every honey material significantly impacts the performance of research participants. Therefore, consuming honey, which is simple to eat and is instantly absorbed by the body, may be a solution for people who have experienced negative effects in the form of excessive post-exercise fatigue. This will help them avoid the negative effects of both light and heavy post-exercise.

CONCLUSION

This study aimed to determine the effect of three types of honey from three different species of honey bees on increasing stamina and anti-fatigue effects. This study shows an increase in the participant's performance in the running after being intervened by consuming three different types of honey (*Apis mellifera*, *Apis cerana*, and *Trigona* sp). In addition, the three types of honey have anti-fatigue effects on the research subjects. However, further research needs to be carried out with higher dose coverage and a more comprehensive range of research subjects, from regular people to professional athletes, to support further the use of honey as an alternative material for recovery.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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