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Phytochemical screening and evaluation of glucose-6-phosphate dehydrogenase activity of fermented honey-garlic in hypothyroidism rats



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ABSTRACT

Introduction: Hypercholesterolemia is an indicator of atherosclerosis in blood vessels and is currently a top priority in overcoming health problems, especially non-communicable diseases in developed and developing countries. Thyroid hormones regulate various metabolic processes in the body including synthesis, mobilization, and lipid degradation. A lack of thyroid hormone in the body or called hypothyroidism will decrease the number of LDL receptors in the liver. This causes an increase in plasma LDL levels which also increases total cholesterol levels. Despite the lack of study, Fermented Honey-Garlic (FHG) is now widely developed for its high antioxidant activity. This study aims to determine the content of bioactive compounds in FHG by qualitative secondary metabolite testing and GC-MS analysis and to evaluate the activity of the enzyme glucose-6-phosphate dehydrogenase (G6PD) in rats with hypothyroidism.

Method: Rats were grouped into 6 groups: positive control group induced by propylthiouracil (PTU) with a dose of 20 mg/ day, negative control treated with distilled water, treatment group with a dose of FHG 0.2 g/kg BW (P1), treatment group with a dose of FHG 0.5 g/kg BW (P2), treatment group with a dose of FHG 1 g/kg BW (P3) and standard drug Simvastatin group with the dose 0,18 mg/day/200 g BW.

Results: Results showed that FHG contained flavonoid compounds, alkaloids, tannins, and saponins, and statistical results showed that the significance value for G6PD activity was 0.014 (P<0.05).

Conclusion: Therefore, it can be concluded that FHG administration yields a significant effect on G6PD levels.

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> overt hypothyroidism than the general population (4.3%)and subclinical hypothyroidism than the general population (11.1%).Cardiovascular disease and mortality were more likely in persons with overt and subclinical hypothyroidism who had serum thyroid stimulating hormone (TSH) levels greater than 10 mLU/L.² Genetic variables are a significant component of the nonmodifiable determinants and earlier epidemiological research, as well as animal models, have revealed that the lack of the glucose-6-phosphate dehydrogenase enzyme may work as a preventive measure against cardiovascular disease (CVD). This enzyme, however, can yield negative physiological effects in response to increased oxidative stress acting as

a cardiovascular risk factor.³ This was according to recent evidence from animal models, ex-vivo studies on cells isolated from deficient subjects, in vitro studies where deficiency was induced by gene silencing, and large human cohorts.⁴

The enzyme (G6PD) aids cells in preventing oxidative stress, which is characterized by an imbalance between enzymatic and nonenzymatic antioxidant defense and the generation of reactive oxygen and nitrogen species (RONS). G6PD performs a variety of tasks, one of which is helping glutathione reductase regenerate reduced glutathione (GSH) from its oxidized version, oxidized glutathione (GSSG). Major endogenous antioxidant GSH defends cells from oxidative damage in several ways. Theoretically, G6PD-

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INTRODUCTION

Lipid metabolism is significantly impacted by thyroid malfunction, which can appear as overt or subclinical hypothyroidism. This results in hypercholesterolemia, which progressively raises the risk of cardiovascular disease and, possibly mortality. Dyslipidemia in hypothyroidism is mostly brought about by an increase in the rate of synthesis rather than breakdown, with the elevated levels of total cholesterol (TC), particularly low-density lipoprotein cholesterol (LDL-C), serving as the substrate for lipid peroxidation by reactive oxygen species (ROS), which causes oxidative stress.¹ According to earlier research, hypercholesterolemic patients have greater prevalences of deficient activity and resulting reduced GSH levels make cells more vulnerable to oxidative stress.⁵ Amino acids, organic materials, vitamins, enzymes, minerals, and aromatics are among the substances found in honey. Flavonoids and phenolic acids, which serve as natural antioxidants, are also in honey.⁶ On the other hand, another natural substance known for its antioxidant properties is garlic.

One of the common uses of single garlic (Allium sativum L. Var. Solo Garlic) in the community is as a seasoning for food and a folk remedy. Single garlic possesses antibacterial, antifungal, antihypertensive, antioxidant, hypoglycemic, and antiaggregation properties. Vitamin C. selenium, allicin, polar phenolic compounds, steroids, essential oils, tannins, alkaloids, saponins, and diallyl disulfide are among the chemicals in garlic that exhibit antioxidant potential.7 Additionally, it is thought that single garlic can help people recover from strokes and infectious infections. Single garlic has a relatively higher herb efficacy than regular garlic. Allicin and saponins, which are the active components of single garlic, have antibacterial properties. Both of these compounds can prevent cholesterol synthesis, which results in blood vessel obstruction.⁸ Honey fermented using single garlic is starting to be widely used but still not much researched. Therefore, this study aimed to determine the content of bioactive compounds in fermented honey-garlic (FHG) and evaluate G6PD levels in hypothyroid rats.

MATERIALS AND METHODS

Materials

The tools used are experimental cages, centrifuge, UV-Vis animal spectrophotometer Sinnowa bs 3000p, micropipette, water bath, Easy Touch GCU (glucose, cholesterol, and uric acid), blood cholesterol strip, blood cholesterol test chip, 3cc syringe. The materials used were forest honey obtained from honey breeders, single garlic (Allium sativum L), propylthiouracil (PTU), and trichloroacetic acid (TCA) 5%.

Data collection procedures

Making fermented honey-garlic (FHG) Fermented honey was made at the Laboratory of the University of Nahdlatul Ulama Surabaya. 8-10 single garlic was crushed and put in a clean glass bottle. It was mixed with forest honey until completely submerged. The mixture was then stored at room temp for 3 days. Honey can be considered fermented well if it released gas every few days. The fermented honey was stored at room temperature.

Analysis of Phytochemical Compound

- a. Tannin Test: Samples of FHG were homogenized with up to 2-3 drops and 3-5 drops of 0.1 percent FeCl₃, and the color changed to a greenishyellow or greenish-brown hue, which was deemed positive (+) for tannin components.⁹
- b. Saponin Test: 10 ml of the FHG was added to 5 ml of distilled water, and the mixture was shaken until foam appeared. 3–5 drops of olive oil were added, and the mixture was shaken again. If there was still foam and it did not go away, the substance was deemed positive (+) for saponin compounds.
- c. Flavonoids Test: FHG sample was added with 1 mL of 10% Pb Acetate solution, and in the second method, the research sample was added with 20% NaOH. The test results obtained showed positive results in both samples after the addition of Pb Acetate was indicated by the formation of a yellow precipitate, while positive results containing flavonoids after the addition of NaOH were indicated by the formation of a yellow solution.
- d. Terpenoid Test: 50–100 mg of FHG was placed on a drip plate, and acetic acid was added until all samples were immersed. This procedure was let to sit for 15 minutes, after which 6 drops of the solution were transferred into a test tube, along with 2-3 drops of concentrated sulfuric acid. The intensity of the resulting color was employed as a comparative indicator of the triterpenoid and steroid content in the sample after the color shifting was observed. The emergence of a reddishorange or purple tint is a sign that triterpenoids are present.¹⁰

e. Alkaloid Test: FHG sample contained alkaloid compounds with the formation of a white precipitate after the addition of the Mayer reagent and an orange precipitate after the addition of the Dragendorff reagent

Preparation of experimental animals

An entirely randomized post-test design was used in this experimental study. The total sample in this study was 30 male Wistar rats and all rats were adapted in the medical laboratory of the Faculty of Medicine of the Universitas Nahdlatul Ulama Surabaya. After the adaptation of the rats to a healthy state, they were then divided into 6 groups based on the treatment to be given, and each group consisted of 6 white rats.¹¹

Treatment of the condition of hypothyroidism

The positive control group (K+), standard drug group, and test group (P1, P2, and P3), were treated with hypothyroidism condition by induction of propylthiouracil (PTU) at a dose of 20 mg/day for 14 days. To determine the condition of the experimental animals, each rat will be examined for blood cholesterol levels using Easy Touch GCU (Glucose, cholesterol, and Uric Acid) by taking blood from the tail of the rat. Rats are considered to have hypercholesterolemia when blood cholesterol levels are > 54 mg/dL. The negative control group (K-) was not given any treatment because conditioned in good health, while the control positive (K+) was not treated with fermented honey so it was assumed as the sick group without treatment. Measurement of cholesterol levels before treatment was carried out after PTU induction for 14 days, while measurements of cholesterol levels after treatment were carried out after treatment groups P1, P2, and P3 were given FHG every day for 14 days.

Standard drug treatment

The standard drug used in this study was simvastatin. The dose used for hypercholesterolemic humans is 10 mg/day. The dose of simvastatin after conversion for white rats (Rattus norvegicus) based on the Laurence and Bacharach conversion table quoted by Haznam (1976) was: 10 mg/day x 0.018 = 0.18 mg/day/200 g BW.

Treatment of fermented honey on experimental animals

Rats indicated for hypercholesterolemia in the test group were treated with fermented honey at three different doses the difference is treatment group with a dose of FHG 0.2 g/Kg BW (P1), the treatment group with a dose of FHG 0.5 g/Kg BW (P2), treatment group with a dose of FHG 1 g/Kg BW (P3) every day for 14 days. A total cholesterol test was performed using Easy Touch GCU.

Measurement of G6PD levels

After treatment, the rats were killed under ketamine injection. The chest and abdomen were smeared with 70% alcohol and surgery were performed to obtain the blood. The blood sample was drawn with a syringe right at the heart and placed in an EDTA tube. Hemolysate is made by 40µl EDTA blood, added by 400µl Pz solvent, and centrifuged at 3000 rpm for 10 minutes. This step was performed in triplicate, then The precipitate was added by 100µl Sol, then stored in the refrigerator at 4°C for 15 minutes. Afterward, it was centrifuged at 3000 rpm for 10 minutes, discarding the supernatant and the precipitate was hemolysate. G6PD levels were calculated using a spectrophotometer at a wavelength of 340 nm.

Data analysis

Analysis of research results from quantitative data presented statistically with SPSS (Statistical Product and Service Solution). The data were analyzed statistically using a non-parametric test, namely by Kruskal Wallis.

RESULT

Analysis of Phytochemical Compound

A phytochemical test is a preliminary test used to identify secondary metabolites in a sample. Phytochemical screening consists of several tests, namely the test for alkaloids, flavonoids, tannins, steroids, and saponins. The results obtained are presented in Table 1 below.

Phytochemical screening results showed that fermented honey garlic

contains alkaloids, flavonoids, tannins, steroids, and saponins

Examination of Cholesterol Levels After FHG Administration

Based on the results of the examination using an Enzymatic photometer, the administration of fermented honey can reduce cholesterol levels in rats as shown in Table 2 below.

In the administration of FHG, the results showed a significant decrease in cholesterol levels in hypercholesterolemic rats as indicated by the results of paired t-test analysis with a p-value of 0.003 (P < 0.05).

Table 1.	Result of	ph	vtochemical	screening

No	Phytochemical screening	Result
1	Alkaloids	+
2	Flavonoids	+
3	Tannins	+
4	Saponins	+
5	Steroids	+

Table 2. Cholesterol level before and after treatment FHG

Groups	Cholesterol Level before treatment (mg/dL)	Cholesterol Level after treatment (mg/dL)	P Value
K-	55.00 ± 10.42	50.00 ± 13.92	
K+	136.75 ± 9.25	62.00 ± 6.71	
P1	131.25 ± 8.53	41.00 ± 5.09	0.002 (D < 0.05)
P2	123.00 ± 5.71	44.75 ± 13.50	0.003 (P<0.03)
P3	117.50 ± 9.57	51.25 ± 10.40	
Std	120.25 ± 6.60	48.75 ± 8.92	

Table 3. Result of G6PD levels

Groups	G6PD levels ± SD (mU/g Hb)	P Value
K-	339.00 ±115.77	
K+	168.00 ± 79.95	
P1	189.67 ± 98.99	$0.014 (D_{<}0.05)$
P2	901.00 ± 519.76	0.014 (P<0.05)
P3	1111.33 ± 745.07	
Std	1230.67 ± 219.76	



Figure 1. Graphical increasing of G6PD levels (mU/g Hb).

Examination of G6PD Levels

Based on the results of the examination using the Sinnowa BS3000P Photometer, the administration of FHG can increase G6PD levels in hypercholesterolemic rats as shown in Table 3 above.

Based on the data in the table above shows that the administration of FHG can increase G6PD levels significantly with a P value of 0.014 (P <0.05).

DISCUSSION

Based on Table 1, the samples of FHG obtained positive (+) results in all tests, namely the test alkaloids, flavonoid tests, tannins, steroids, and saponins. To determine the effect of the fermentation process on the content of secondary metabolite compounds in forest honey samples in this study, a qualitative test was carried out namely phytochemical screening. This test consists of several assays, namely alkaloids, flavonoids, tannins, steroids, and saponins. The alkaloid test was carried out using two types of reagents, namely Mayer's reagent and Dragendorff's reagent. The test results obtained showed that the sample contained alkaloid compounds with the formation of a white precipitate after the addition of the Mayer reagent and an orange precipitate after the addition of the Dragendorff reagent. The principle of this analytical method is the precipitation reaction that occurs due to a reaction between nitrogen atoms which has a lone pair of electrons of the alkaloids that can replace with iodine ion. Positive results for alkaloids in Mayer's test are indicated by the formation of a white precipitate, which is a potassium-alkaloid complex. The nitrogen in the alkaloid is predicted to react with the metal ion K+ from potassium tetraiodomercurate(II) in Mayer's reagent alkaloid test to generate a precipitated potassium-alkaloid complex. Dragendorff's test for alkaloids showed positive results when a light brown to yellow (orange) precipitate formed. The precipitate is a potassium alkaloid. In the alkaloid test with Dragendorff's reagent, nitrogen is used to form a coordinate covalent bond with K⁺. In the flavonoid test on FHG samples, tests were carried out using 2 types of reagents, namely Pb Acetate and NaOH. In the first method,

the FHG sample was added with 1 mL of 10% Pb Acetate solution, and in the second method, the research sample was added with 20% NaOH. The test results obtained showed positive results in both samples after the addition of Pb Acetate was indicated by the formation of a vellow precipitate, while positive results containing flavonoids after the addition of NaOH were indicated by the formation of a yellow solution. In the tannin test, the FHG sample was tested using FeCl, reagent to determine whether there was a phenol group in the sample. The presence of a phenol group is indicated by a blackish-green or dark blue color after being added with FeCl.. The results of this study obtained positive results which were indicated by the formation of a dark green or blue color which indicated the formation of a complex compound between tannins and Fe which indicated a strong green, red, purple, blue, or black color change. The steroid test on the FHG sample was based on the ability of the steroid compound to form a color with concentrated H₂SO₄ in a hydrochloric acid solvent. The results showed positive results which were indicated by the formation of a blue/greenish-blue ring. A glycolic acid is a polar group that faces outward in saponins, whereas steroid and triterpenoid groups are nonpolar groups that face inward. When shaken with water to create foam, both of these groups can produce micelles because they are both active on the surface. The presence of glycosides with the capacity to create foam in water, which is digested into glucose and other compounds, is indicated by the presence of foam.¹² The research by Poernomo and Ma'ruf (2020) found that the phytochemical screening test on garlic produced positive results containing active components, such as flavonoids, alkaloids, phenolics, and tannins. The data from this research are in line with past research.¹³

This study discusses the results of the test to prove the effect of FHG on Glucose 6 Phosphate Dehydrogenase (G6PD) levels in a hypothyroid state which is characterized by high cholesterol/ hypercholesterolemia levels. Based on the results in Table 1 the average high cholesterol levels were observed in the positive control group, this it can be said that the rats had hypothyroidism. This occurs because the administration of PTU inhibits thyroid hormone synthesis. Thyroid hormone induces an increase in the number of LDL receptors, therefore cholesterol levels increase.¹⁴ Previous research explained that administration of Rosa piriformis fruit extract increased the activity of Glucose 6-phosphate dehydrogenase (G6PD) significantly compared to the control (p<0.001). This study concluded that this extract may reduce the inhibitory impact of streptozotocin on enzyme activity.¹⁵

Based on the results in Figure 1 shows that the higher the dose of fermented honey, the higher the levels of G6PD. Fermented honey contains ingredients derived from single garlic and honey. Honey contains chemical compounds that act as antioxidants, namely phenolic acids and flavonoids. The main composition of phenolic acids and flavonoids in honey consists of coumaric acid, isorhamnetin, chrysin, quercetin, galangin, luteolin, ellagic acid, gallic acid, syringic acid, benzoic acid, cinnamic acid, ferulic acid, myricetin, chlorogenic acid, caffeic acid, hesperetin, and cinnamic acid.¹⁵ These antioxidant properties can reduce the effect of intracellular ROS and stabilize G6PD levels. Single garlic is one of the spices that is believed to have many benefits, one of which is having antioxidant properties. The polyphenol content found in single garlic can be directly related to antioxidant activity.¹⁶ Antioxidant substances such as flavonoids and polyphenols are known to increase the production of G6PD.¹⁶ Based on table 3 showed that there is a significant difference between all of the groups on G6PD levels with the P value was 0.014 (P<0.05). It means that FHG has a significant effect in increasing G6PD levels because of its bioactive compounds such as flavonoids, alkaloids, tannins, steroids, and saponin.

CONCLUSION

Based on the results on G6PD levels in hyperthyroid rats that were administered with FHG, the P value was 0.014 (P<0.05). It can be concluded that the administration of FHG has a significant effect on G6PD levels because of its bioactive compounds such as flavonoids, tannins, alkaloids, steroids, and saponins.

AUTHOR CONTRIBUTION

All authors contributed to this research starting from the stage of conception and design, collection, analysis, and interpretation of data, preparation of articles, submission, and article revision, as well as proofreading article.

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CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this investigation.

ETHICAL CONSIDERATION

Universitas Nahdlatul Ulama Surabaya's Ethics Council for Health Research has deemed this study to be ethical with the number 102/EC/KEPK/UNUSA/2022.

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Achmad Lefi, Lalu Galih Pratama Rinjani, Muhammad Yusuf Alsagaff, Raden Mohammad Budiarto, Priangga Adi Wiratama, Etty Hary Kusumastuti, Isnin Anang Marhana, Alfian Nur Rosyid, Dwi Wahyu, Bambang Pujo Semedi, Ricardo Adrian Nugraha, Ryan Ernast Intan, Muhammad Ramadhan, Ika Caesarina, Asiyah Nurul Fadila, Muhammad Surya Tiyantara, Gilang Muhammad Setyo Nugroho, Ummi Maimunah, Edi Suyanto, Adhitri Anggoro, I Komang Rusgi Yandi, Jilieanastasia Godrace Lilihata
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Yasi Anggasari, Fauziyatun Nisa, Ratna Ariesta Dwi Andriani, Yunik Windarti

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Marisa Surya Airlangga, Reni Prastyani, Nurwasis, Hani Plumeriastuti, Budi Utomo		
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The role of mean platelet volume (MPV) and platelet distribution width (PDW) pre-operating as sepsis indicators in laparotomy		
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