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Anti-diabetic and anti-cholesterol activity of *Kaempferia galanga* L. herbal medicine rhizome in albino rats

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Abstract
Kaempferia galangal is an herb commonly used as a kitchen spice, herbal medicine for maintaning vitility, body detox, diarrhea medication, treat arthritis, cough medicine, influenza medicine, etc. The purpose of this study was investigate role of anti- diabetic and anti-cholesterol activity of *Kaempferia galanga* herbal medicine rhizome on albino rats. The design used in this study was Completely Randomized Design with the treatment of different dose of *Kaempferia galanga* herbal medicine rhizome namely: P0 (Control/without *Kaempferia galanga* herbal medicine), P1 (dose of 150 mg/kg body weight *Kaempferia galanga* herbal medicine), P2 (dose of 200 mg/kg BW *Kaempferia galanga* herbal medicine), P3 (dose of 2500 mg/kg BW *Kaempferia galanga* herbal medicine), and P4 (dose of 300 mg/kg BW *Kaempferia galanga* herbal medicine), repectively. Treatment of *Kaempferia galanga* herbal medicine is done by gavage method everyday for 30 days. The parameters observed were glucose and rat blood cholesterol levels before and after *Kaempferia galanga* herbal medicine rhizome treatment. The results showed that rat glucose levels before treatment was non significant differences (P>0.05), and after *Kaempferia galanga* rhizome herbal medicine treatment between control and treatment showed significant differences (P<0.05). Cholesterol levels of *Kaempferia galanga* rhizome herbal medicine treatment were non significantly different (P>0.05) between control and treatment rats. It was conculded that *Kaempferia galanga* rhizome herbal medicine has potential as anti-diabetic medicine on certain doses, but not potential as anti-cholesterol medicine.

Keywords: *Kaempferia galanga*, rat, glucose, cholesterol

Introduction
Kencur or its scientific name *Kaempferia galanga* L. is an herb with many benefits. Traditionally the rhizome of this plant has long been used as a kitchen spice and a mixture of herbs to maintain body vitality, play arole as body detox, diarrhea medication, treat arthritis, cough medicine, flatulence, influenza and so on. Kencur rice herbal medicine is one of the herbs that is very popular with the people of Indonesia to maintain the vitality of the body. Kencur traditional ingredients mixed with salt have long been known as phlegm cough medicine so that breathing becomes more relieved. Harini and Soekarman (2012) [13] say that out of 130 herbs, about 20 herbs contain kencur, 7 for women's health care, 2 kinds for refreshment, 2 for blood circulation, 9 for strengthening the body. Kencur is usually combined with other herbs such as turmeric, ginger, honey as ready-to-drink herbal drinks (Pudjiastuti *et al.*, 2015) [25].

Several scientific studies have been conducted to prove the efficacy of the efficacy of *Kaempferia galanga* rhizomes such as; the results of research by Liana *et al*. (2012) [18] said that white rats induced by acetosol to make stomach irritation and treated with *Kaempferia galanga* rhizome, erosion of rat gastric mucosa can be treated and returned to normal. Hasanah *et al*. (2011) [15] and Hendriati *et al*. (2010) [16] say that kencur rhizome has the potential as an anti-inflammatory drug and is anti-bacterial (Masniari *et al.*, 1996) [19]. The results of Ramadan's (2016) [26] showed that the administration of kempur (*Kaempferia galanga*) rhizome infusion caused decreased motility of male mice (*Mus musculus*) along with increasing doses of Kencur. Chotimah *et al*. (2014) [5], making candles from *Kaempferia galanga* rhizome as aromatherapy and pharmacotherapy that makes the feeling of being more relaxed and reducing the use of chemicals for aroma therapy. Handayani (2015) [9] reported that Kencur rhizome has the potential to prevent osteoporosis and cholesterol-lowering

through *in-vivo* and *in-plico* studies. Turmeric mixed with galingale can also be used as a traditional scrub material and the quality is getting better (Arbani *et al*., 2015) [2]. Kencur rhizome also has the potential as a sunscreen preparation, even though its unstable power is not stable (Widji *et al*., 2012) [30], and Kencur rhizome as an analgesic /pain relief drug (Wuryaningsih *et al*., 1996) [32]. Haniastuti (2009) [11] reported that there was a 1β interlinine synthesis of mice macrophage cells induced by *E. coli lipopolysaccharide* and Kencur essential oil which was carried out *in vitro*. Kencur rhizome is an anti-inflammatory agent by inhibiting the enzyme cyclooxygenase 1 and 2 (Umar *et al*., 2016) [29]. Kencur rhizome extract is also potential as an antifungal by inhibiting the growth of *Candida albicans* (Annisa *et al*., 2016) [1]. Flavonoid compounds found in Kencur are immunomodulated against microbial abilities of neutrophil cells (Revilla *et al*., 2008) [27]. However, not all bacteria can be eliminated by the active substance contained in the *Kaempferia galanga* rhizome as the results of Nugraha *et al.* (2012) [22] study that the growth of ethyl p-methoxycinamate and Kencur extract did not have the growth inhibition activity of *Bacillus subtilis* bacteria.

From the results of chemical analysis of the methanol extract of galingale rhizome with the GCMS method it was found that the Kencur rhizome contains approximately 23 compounds and 17 of them are aromatic compounds (essential oils) (Chairul *et al.*, 1996) [4]. Essential oils found in Kencur rhizome are approximately 0.6% p-methoxisinnamate ethyl ester. The six main compounds found in *Kaempferia galanga* rhizome are glycolic acid, bomeol, ethyl sinnamat, 6-profile tridecane, m-methoxy sinnamate ethyl ester, p-methoxy sinnamate ethyl ester and 18 other minor compounds (Haripini *et al*., 2012) [14]. Results of the research Ekawoti *et al.* (2015) [7] said that Ethyl p-methoxycinnamate contained in the Kaempferia galanga has the potential as an angiogenesis inhibitor through the tyrosin kinase enzyme. But if the dose of kaempferol given is too large, this can also interfere with the body's physiological system. The results of the study by Pudjiastuti *et al.* (1996) [24] said that the greater the dose of galingale, the greater the abortive nature, followed by the lighter weight of fetal white rats.

Normal glucose levels in the blood are very important to keep the body working and functioning properly. Glucose is needed by cells as fuel to produce energy. If the body lacks glucose, the body will not be able to work optimally, which is characterized by a weak, pale, difficult concentration, and so on. High glucose levels continuously in the blood, will also cause health problems such as diabetes, high blood pressure, kidney and so on. Normal glucose levels in humans around 70-100 mg/dl after fasting approximately 8 hours. These levels can change after eating, sleeping or in a state of fasting. Blood glucose levels are also strongly influenced by the type of food that enters the body, such as several types of substances that are anti-diabetes which can reduce blood glucose levels including breadfruit leaves (Dipa *et al*., 2015) [6]. Parisa and Siamak (2010) [23] reported that consumption of vitamin C (ascorbic acid) over a long period of time caused the treatment rats to experience hyperglycemia (diabetes) and also an increase in weight loss. Latifah (2014) [17] reported that Kencur rice has antidiabetic activity as indicated by a decrease in blood glucose levels, weight control, as well as an improvement in the pancreatic Langerhans islands of rats induced by streptozotocin with an effective dose of 1.9 mL/200 g body weight.

Like glucose, cholesterol in the blood is also very important. Cholesterol is needed by the body for components of cell walls, as precursors of steroid hormones and others. However, high cholesterol levels in the body are closely related to high blood pressure, blood vessel constriction, coronary heart disease, stroke and so on. Blood cholesterol levels are strongly influenced by food intake that enters the body (Budiman *et al.*, 2016) [3]. Foods that contain trans fats, fried, offal will usually increase blood cholesterol levels. Foods that contain lots of fiber, acids usually will reduce cholesterol levels in the blood. Handayani (2015) [9] said that Kencur is anti-cholesterol which can reduce blood cholesterol and LDL levels.

Materials and Methods

The design used in this study was a completely randomized design with the treatment of different doses of Kencur juice drinks and 10 replications so that the experimental animals used were 50.

Research material

The main ingredient used in this study was the Kencur rhizome (*Kaempferia galanga* L.) which was made into dried simplicia and then blended into fine powder. Kencur dose used refers to Handayani (2015) [9]. Kencur dried rhizome powder was weighed according to the dosage and dissolved with water, then given to rats by 1 ml of gavage (stroke) method. The experimental animals used were adult male rats aged 2-3 months with a body weight between 100-125 grams.

Research procedure

The research procedure can be seen in the research flow chart. A total of 60 male rats aged 3–4 months measured their initial body weight, initial cholesterol levels and final cholesterol levels. Rats were given a standard formula diet and drank water *ad libitum* and acclimatized for 4 weeks, then the rats were divided randomly into 6 groups: K = control (rats were given boiled water); P1 = rat group + 150 mg dose of Kencur rhizome; P2 =, group of mice + 200 mg dose of Kencur rhizome; P3 =, rat group + 250 mg dose of Kencur rhizome; P4 =, group of mice + 300 mg dose of hamferia galangal L rhizome. Before being treated with Kencur juice drink, rats were acclimatized in a trial cage for 1 week. After that, then treated with ginger juice drink according to the treatment dose. The treatment of galingale juice is done orally by gavage method according to the dosage using a needle sonde. The treatment is carried out every day for 30 days. After the treatment is complete, testing cholesterol levels and blood glucose levels, taking the pancreas by dissecting it, then the pancreas is cleaned with 0.9% NaCl and put into 10% NBF fixative solution for making histological preparations.

Testing of glucose levels and cholesterol levels

Rat blood glucose and blood cholesterol levels were tested with the Nesco multiple check tool. Glucose and blood cholesterol levels of the rats tested were the initial blood glucose and cholesterol levels before they were treated with Kencur juice drink, where previously the rats were fasted (not fed) for approximately 8 hours. After that the blood is taken from the tail vein, by cutting off the tip of the tail with sterile scissors that has been smeared with alcohol, the blood coming out from the tip of the tail is then dripped on the stick for

testing, for further reading by the Nesco multiple check tool. Wounds that arise after the tail is cut are treated with betadin. Then the test is carried out every two weeks after treatment until the end of the treatment.

Results

Initial blood glucose levels analyzed by variance analysis did not show significant differences (P= 1.00), whereas final blood glucose levels showed significant differences (P= 0.011) (Table 1). There were significant differences in blood glucose levels between control rats and P1 and P3 rats. There was a tendency to decrease rat blood plasma glucose levels after receiving treatment for 30 days of *Kaempferia galanga* rhizome extract.

Table 1: ANOVA assay and standard error of initial and final glucose levels of male rats (*Rattus novergivus* L.) supplemented with *Kaempferia galanga* herbal medicine allowed by Duncans test

No	Treatment	Average of initial blood glucose level (mg/dl)	Average of final blood glucose level (mg/dl)
1	C (control)	102.20 ± 8.66a	108.00 ± 3.30ab
2	P1 (dose of 150 mg/kg Body Weight)	103.00 ± 9.93a	96.40 ± 4.26bc
3	P2(dose of 200 mg/kg Body Weight)	102.20 ± 8.66a	109.60 ± 2.76a
4	P3 (dose of 250 mg/kg Body Weight)	103.00 ± 9.93a	95.000 ± 4.48c
5	P4 (dose of 300 mg/kg Body Weight)	102.20 ± 8.66a	115.00 ± 5.55a

Initial blood cholesterol levels analyzed by variance analysis did not show significant differences (P = 0.11), while the results of analysis of final blood cholesterol levels analyzed by variance analysis also did not show significant differences (P= 0.2 64) (Table 2). Between control rats and P3 treatment rats there was a decrease in the average cholesterol level, but with a large standard of error which means the data is very diverse.

Table 2: ANOVA assay and standard error of initial and final cholesterol levels of male albino rats (*Rattus novergivus* L.) supplemented with *Kaempferia galanga* herbal medicine allowed by Duncans tes

No	Treatment	Average initial blood cholesterol level (mg/dl)	Average final blood cholesterol level (mg/dl)
1	C (control)	187.20 ± 17.89a	182.40 ± 13.7a
2	P1 (dose of 150 mg/kg Body Weight)	144.20 ± 13.26a	169.40 ± 9.06a
3	P2 (dose of 200 mg/kg Body Weight)	153.40 ± 13.65a	150.40 ± 7.74a
4	P3 (dose of 250 mg/kg Body Weight)	191.20 ± 11.06a	155.20 ± 14.10a
5	P4 (dose of 300 mg/kg Body Weight)	187.20 ± 17.89a	186.00 ± 18.84a

Discussions

The level of glucose in the blood varies greatly because it is influenced by several things such as; post absorption, hormonal imbalances, genetic disorders, and diet. Blood glucose levels depend on hormones released by the adrenal glands, namely adrenaline and corticosteroids and the hormone insulin released by the pancreas. Adrenaline will increase blood glucose, while corticosteroids will decrease. Adrenaline which is driven continuously will result in insulin not being able to regulate ideal blood glucose levels so that blood glucose levels increase (Latifah, 2014) [17]. In addition, drugs or foods that contain antidiabetic substances can also reduce blood glucose levels such as Dutch teak leaf extract and extract of temu lawak, ginger extract (Guan-Ling *et al*., 2015) [8]. Glucose levels measured are usually fasting blood glucose levels.

Decrease in blood glucose levels in rats treated with *Kaempferia galanga* rizhome herbal medicine may be caused by flavonoids which are as immunomodulative agents that can improve the body's defense system of rats (Revilla *et al.*, 2008) [27], in this case flavonoids are anti-oxidants that repair damage to pancreatic cells as insulin-producing cells caused by streptomycin induction. Flavonoids contained in Kencur rhizome can reduce blood glucose levels in certain doses or in other words Kencur has the potential as an anti-diabetes drug. The phytochemical content of essential oils such as steroids, triterpinoids and resins contained in *Kaempferia galanga* rhizome also have the potential as anti-diuretic drugs as said by Mohamad *et al.* (2006) [21]. But if the levels of kaemferol given too large can also interfere with the physiological function of the body as it can cause glucose levels to rise, or in other words it can have a negative effect. Similar thing was expressed by Pudjiastuti *et al.* (1996) [24] that high levels of kaempferiol cause miscarriage in fetal mice and a decrease in fetal weight of rats. Hanif *et al*. (2018) [12] and Guan-Ling *et al.* (2015) [8] said that *Kaempferia galanga* also has the potential as an anti-cancer (killing cancer cells) because of its anti-oxidant content, even though its mechanism of action has not been revealed. Antioxidants contained in kencur like essential oils, ethyl p-methoxy cinnamate has been successfully produced in vitro by Suprava *et al.* (2014) [28] so that exploitation of the use of *Kaempferia galanga* from nature for various treatment needs can be reduced.

Pancreatic histology between control rats and rats treated with Kencur rhizome extract showed abnormalities such as: fat degeneration, beta cells experienced necrosis, but very little percentage. Every healthy organ, there are still some cells that are damaged or dead, but there are not so many that they do not interfere with the physiological function of the organ. The size of the Langerhans island diameter (PL) when compared between control rats and treatment rats, looks different is not real. The treatment of Kencur rhizome juice in rats reduced total cholesterol levels in rats' blood. However, this decrease in cholesterol levels between control and treatment did not show significant differences. This is contrary to what was stated by Wirapati (2008) [31] that chicken boilers that were fed with Kencur flour cholesterol levels in their meat decreased. One of the factors that influence blood cholesterol is the content of crude fiber ration because coarse fiber will fill the bile acids in the digestive tract and then expelled together with feces. This condition will reduce fat absorbed into the body and reduce bile acid synthesis from cholesterol. As a result total cholesterol in the body will decrease. Bile acids are synthesized from cholesterol and act as emulsifying

agents which prepare the triacylglycerol ration before hydrolyzing the pancreatic lipase enzyme in the digestive process (McDonald *et al.*, 2002) [20].

Conclusions

We conclude that the rat blood glucose levels decreased after receiving treatment with *Kencur* rhizome herbal medicine in P1 treatment (150 grams/kg body weight) and P3 (250 grams/kg body weight. Herbs of Kencur rhizome have the potential as anti-diabetic drugs at certain doses but do not have the potential as anti-cholesterol drugs.

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