p-ISSN 2615-787X e-ISSN 2615-790X
Accredited by Directorate General of Higher Education, Research, and Technology, Republic of Indonesia, No. 225/E/KPT/2022

Tropical Animal Science Journal, December 2023, 46(4):494-501
DOI: https://doi.org/10.5398/tasj.2023.46.4.494
Available online at https://journal.ipb.ac.id/index.php/tasj

Antidiabetic and Hypolipidemic Potential of Cow's Milk Yogurt with the Addition of Herbal Extracts in Diabetic Rats

D. F. Pazra[a,*], M. Purwanti[a], K. S. Handayani[a], Wahyuningsih[a], & S. Wahyuwardani[b]
[a]Animal Health Division, Bogor Agricultural Development Polytechnic
Bogor-West Java, Indonesia
[b]Research Center for Veterinary Science, National Research and Innovation Agency
Bogor-West Java, Indonesia
*Corresponding author: debbyfadhilah99@gmail.com
(Received 12-06-2023; Revised 11-08-2023; Accepted 14-08-2023)

ABSTRACT

Yogurt, with the addition of herbal ingredients can add efficacy and value of its benefits. Several studies have proven that cinnamon, aloe vera, and fenugreek seeds have properties as antidiabetic and hypolipidemic. The purpose of this study was to evaluate the antidiabetic and hypolipidemic potential of yogurt with the addition of a combination of two or three types of herbal extracts, such as cinnamon, fenugreek seeds, and aloe vera, in diabetic rats. Diabetic rats were divided into six groups and given treatment for 21 days. Rats' blood was taken to test blood glucose levels using glucometers, insulin with the ELISA method, blood lipid profiles, and kidney and liver functions using a chemistry analyzer. The data were statistically analyzed using one-way ANOVA followed by the Duncan test. The results showed that yogurt added with several combinations of herbal extracts such as cinnamon, aloe vera, and fenugreek seeds (III, IV, V, and VI) could significantly reduce blood glucose levels, increase insulin levels, improve lipid profiles, and improve kidney and liver functions in diabetic rats, as well as commercial administration of glibenclamide (II). Diabetic rats given yogurt with the addition of a combination of 3 herbal extracts (VI) and a combination of 2 herbal extracts (cinnamon +fenugreek seeds) (V) of 1% each showed better antidiabetic and hypolipidemic performances compared to the other groups (III, and IV). The combination of yogurt with some herbal extracts can add efficacy and value to the benefits of yogurt as a functional drink.

Keywords: aloe vera; cinnamon; diabetes; fenugreek seeds; yogurt

INTRODUCTION

Diabetes is a metabolic disorder that affects the body on a long-term basis and is characterized by endocrine abnormalities and persistent hyperglycemia (American Diabetes Association, 2018; Abdul *et al.*, 2020; Kottaisamy *et al.*, 2021) that occurs when the level of glucose in a person's blood increases because his body cannot produce enough insulin hormone or cannot use the insulin it produces effectively (Westman, 2021). The persistent and varied symptoms of hyperglycemia include abnormalities in carbohydrates, fats, and proteins metabolisms. Abnormalities in either insulin secretion or insulin action or both cause hyperglycemia. Diabetes presents in various ways and progresses in a complex manner with a complex pathophysiology (American Diabetes Association, 2014). The primary mechanism by which hyperglycemia contributes to the onset and progression of these complications is the production of reactive oxygen species (ROS), which causes lipid peroxidation and membrane damage. Cardiovascular (CV) risk factors such as obesity, hypertension, and dyslipidemia are common in diabetic patients, increasing their risks for cardiac events (Al Kury *et al.*, 2022). Type 2 diabetes (T2DM) is the most common form of diabetes in about 80% of all diabetic populations and is characterized by what is known as insulin resistance (Chaudhury *et al.*, 2017).

An estimated 463 million people had diabetes in 2019, and this number is expected to increase to 578 million by 2030 and 700 million by 2045 (Cho *et al.*, 2018; Saeedi *et al.*, 2019). The prevalence of diabetes in Southeast Asia was ranked third in the world in 2019. While Indonesia itself is ranked as the 7th country with the highest prevalence of diabetics in the world and the only Southeast Asian country that is included in the top 10 (Ligita *et al.*, 2019).

One of the functional foods that has recently been in demand by the public is yogurt. Yogurt consumption is associated with a reduced risk of type 2 diabetes. This is due to the high content of calcium, magnesium, vitamin D, whey protein, and certain fatty acids in dairy products. Some studies show that whey protein has insulinotropic and glucose-lowering properties (Chen *et al.*, 2014). In addition to these ingredients, other ingredients in yogurt that also have an antidiabetic role

are probiotics. Research by Zeng *et al*. (2016) shows that *Lactobacillus lactis* probiotic bacteria have the potential to be applied as antidiabetic probiotics because of their ability to inhibit the enzyme -glucosidase so that it can reduce carbohydrate absorption in the intestine.

Previous research has proven that yogurt-added natural ingredients (herbs and spices) can add efficacy and value benefits and diversify food products (Dabija *et al.,* 2018). Lately, yogurt is often served with the addition of natural ingredients such as cinnamon (*Cinnamomum burmannii*), aloe vera (*Aloe vera*), and fenugreek (*Trigonella foenum-graecum* L.) seeds. We find these three herbal ingredients in Indonesia as nutritional and health supplements. Several studies have proven some of these herbal ingredients (cinnamon, aloe vera, and fenugreek seeds) have antidiabetic and hypolipidemic properties because they have high antioxidant content such as flavonoids, saponins, tannins, and alkaloids (Belguith-Hadriche *et al.,* 2013; Kusumaningtyas *et al.,* 2014; Samaneh *et al.,* 2015; Beji *et al.,* 2018). Bioactive components mainly terpenoids, alkaloids, flavonoids, and other types of NPs, which exhibit *in vivo* hypoglycemic effects (Ma *et al.,* 2022).

Based on the description above, yogurt with the addition of several combinations of herbal extracts such as cinnamon, aloe vera, and fenugreek seeds, has the potential to be an antidiabetic functional drink. Still, so far, there has been no *in vivo* testing with experimental animals. Therefore, it is necessary to conduct this study, which aims to evaluate the antidiabetic and hypolipidemic potential of yogurt with the addition of a combination of two or three types of herbal extracts such as cinnamon, fenugreek seeds, and aloe vera in diabetic rats.

MATERIALS AND METHODS

Herb Extraction

The herbal extracts that were used in this study were cinnamon (*C. burmanii*), fenugreek (*T. foenum-graecum* L.) seeds that were purchased from a local supplier in West Java, and aloe vera taken from the practice fields of the Bogor Agricultural Development Polytechnic. The process of herbal extraction used the maceration method with 70% ethanol solvent. Dried cinnamon, fenugreek seeds, and aloe vera were ground into flour, then weighed and extracted with 70% ethanol at a ratio of 1:10 by maceration on an orbital shaker at 28 °C for five days. The extract was filtered and evaporated using a vacuum evaporator at a temperature of 40 °C to produce a thick extract. After that, the herbal extracts were stored until the next process (Salehi *et al.,* 2013).

Yogurt Preparation

This study used the Bi-Proyo yogurt starter culture from the Indonesian Center for Agricultural Post Harvest Research and Development. Bi-Proyo yogurt starter culture contains *Lactobacillus bulgaricus* ENCC 0041, *Streptococcus thermophillus* ENCC 0040, *Bifidobacterium longum* ATCC 15707, and *Lactobacillus casei* FNCC 0090. A freeze-dried culture of 6.5 g was dissolved in 50 mL of pasteurized cow's milk, then incubated for 24 hours at 28 °C. Furthermore, the mixture was added to 1 liter of pasteurized cow's milk and incubated for 24 hours at 28 °C. The yogurt formed was stored at a cold temperature (4 °C) and used as a starter culture (Juniawati *et al.,* 2020).

Cow's milk was heated for 30 minutes at 70-80 °C, then inoculated with 4% starter culture when the temperature was cooled down to approximately 40 °C. After that, it was incubated for 24 hours at 28 °C and fortified with 1% herbal extract. Yogurt was stored at a cold temperature (4 °C) (Juniawati *et al.,* 2020). This study added several combinations of herbal extracts to yogurt, i.e., cinnamon + fenugreek seeds extract, cinnamon + aloe vera extract, fenugreek seeds + aloe vera extract, and a combination of cinnamon + fenugreek seeds + aloe vera extract.

Design of Experimental Study

This study used experimental animals, male Sprague-Dawley rats aged 11 weeks weighing 200 g–230 g obtained from the Center for Veterinary Research, Bogor. This study was conducted by following the National Research Council: Guide for the Care and Use of Laboratory Animals (National Research Council, 2011). Ethical permits for research using experimental animals were issued by the Ethics Committee of the Agricultural Research and Development Agency with Ethical Approval Number: Balitbangtan/BB Litvet/Rd/02/2020. Animals were maintained under standard laboratory conditions, namely rat cages set at a temperature of 22–25 °C, a humidity of 55%–63%, and light settings of 12 hours bright and 12 hours dark. Experimental animals were given standard feed that refers to Nutrient Requirements of Laboratory Animals (National Research Council, 1995) and drank ad libitum. Rats were first given anthelmintics and acclimatized for 7 days.

A total of 30 male Sprague-Dawley rats were divided into six treatment groups, with each group consisting of five rats. After the rats were acclimatized, the experimental rats were satisfied for 12 hours, and baseline blood glucose measurements were taken. After that, an intraperitoneal injection of streptozotocin (STZ) was carried out, which was dissolved in 0.1 M citrate buffer, pH 4.5, at a dose of 45 mg/kg BW. The condition of diabetes in rats was confirmed by checking blood glucose levels for three consecutive days to ensure persistent hyperglycemia in rats. Rats with blood glucose levels >200 mg/dL were grouped and given oral treatment (sonde) for 21 days.

The group of experimental rats in this study was divided as follows: 1.) Diabetic rats without treatment and only given distilled water orally as a positive control (I); 2.) Diabetic rats were given a commercial drug (glibenclamide) orally at a dose of 10 mg/kg body weight as a negative control (II); 3.) Diabetic rats with the administration of cow's milk yogurt + 1% aloe vera extract + 1% fenugreek seed extract (III); 4.) Diabetic rats with cow's milk yogurt + 1% cinnamon extract + 1% aloe vera extract (IV); 5.) Diabetic rats with the administration of cow's milk yogurt + 1% cinnamon extract + 1% fenu-

greek seed extract (V); 6.) Diabetic rats with cow's milk yogurt + 1% cinnamon extract + 1% aloe vera extract + 1% fenugreek seed extract (VI). Yogurt with the addition of several combinations of herbal extracts was given orally with dose determination based on allometric scaling comparison with dose comparison in humans and rats, where the effective dose of yogurt reduced several criteria in metabolic syndrome patients by 125 mL/day (Kaminskas *et al.*, 2013) so that the dose of administration was 3.4 mL/200 g BW.

Weight measurement and blood collection through the tail vein were carried out on the 7th, 14th, and 21st days after the treatment to look at the blood glucose levels of the rats, while for testing the level of insulin in the blood, the biochemical profile of the blood was carried out after the 21st day. Measurement of blood glucose levels using a glucometer (EasyTouch GCU).

Serum insulin was measured by ELISA technique using a rat insulin ELISA kit (E-EL-R3034, Elabscience, USA). A total of 100 µL of standard solution, blanks, and samples were added to the well plate (well) and then covered with a sealer. Incubation was carried out for 90 minutes at a temperature of 37 ^{o}C. The solution in each well was discarded (not washed), and then each well was added 100 µL of Biotinylated Detection Ab working solution. The plate was then covered with a sealer and incubated for 1 hour at a temperature of 37 ^{o}C. The solution in each well was removed, and then 350 µL of wash buffer was added to each well. The wash buffer was allowed to stand for 1-2 minutes, then removed and dried with absorbent paper. Washing with a wash buffer was repeated three times. Each well was added 100 µL HRP conjugate working solution and covered with a sealer, then incubated for 30 minutes at 37 ^{o}C. The solution in each well was removed, and the washing process with a wash buffer was carried out again five times. Substrate reagent was added to each well, then covered with a sealer and incubated for 15 minutes at 37 ^{o}C. The plate was kept away from the light. A total of 50 µL stop solution was added to each well, which was then read at λ 450 nm with an ELISA reader.

Blood plasma was required for testing the biochemical profile of blood obtained through a blood sample that was taken and inserted into a vascular tube, then centrifuged at a speed of 2500 rpm for 15 minutes. After that, the blood plasma was separated and inserted into a microtube. The biochemical profile of blood measured includes total cholesterol (TC), triacylglycerol (TG), high-density lipoprotein cholesterol (HDL), low-density lipoprotein-cholesterol (LDL), urea, creatinine, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) using a chemistry analyzer (Selectra Junior Analyzer, USA) and the reagent kit of Roche Diagnostics GmbH, Mannheim (Germany).

Statistical Analysis

Statistical analysis was used one-way ANOVA followed by Duncan's further test using SPSS software. A value of $p<0.05$ was considered statistically significant. The results of the analysis were presented in the form of tables as the average value ± standard deviation.

RESULTS

Body Weight Measurement

Body weight measurements in experimental rats showed an increase every week in the group of diabetic rats given treatment, while the group of diabetic rats without treatment showed a decrease every week. There was a significant difference in the body weights of diabetic rats between the treatment groups (II, III, IV, V, and VI) and the non-treated group (I), especially on the 21st day after treatment. Still, there was no significant difference between the treatment groups that were given yogurt with the addition of some combination of herbal extracts. The results of body weight measurement in experimental rats can be seen in full in Table 1.

Glucose and Insulin Levels

The results of checking blood glucose levels in experimental rats showed an increase, which was detected 5 days after treatment (Table 2.). Blood glucose levels began to decrease from day 7 to day 21 after treatment. The blood glucose levels of the experimental rats at 21 days after treatment were significantly different between the untreated group of diabetic rats (I) and the treated group (II, III, IV, V, and VI). Glucose levels be-

Table 1. Average body weight in the group of experimental rats

Group	Average body weight (g)			
	H0	H7	H14	H21
I	227.20± 5.63^{a}	220.60± 4.67^{a}	218.00± 4.64^{a}	216.60± 6.58^{a}
II	218.80± 0.84^{a}	223.00± 1.00^{ab}	230.60± 1.95^{ab}	241.20± 3.90^{b}
III	223.20± 7.19^{a}	226.00± 8.15^{ab}	231.60± 7.02^{ab}	239.40± 8.20^{b}
IV	234.40±20.89^{a}	238.60±20.72^{b}	244.00±20.65^{b}	251.20±20.89^{b}
V	226.60±11.95^{a}	231.00±11.90^{ab}	237.20±12.32^{b}	244.40±12.48^{b}
VI	227.20±13.83^{a}	232.20±14.45^{ab}	238.40±14.59^{b}	246.80±15.06^{b}

Note: Means in the same column with different superscripts differ significantly ($p<0.05$). HO= Day before treatment; H5= 5th day post-treatment; H7= 7th day post-treatment; H14= 14th day post-treatment; H21= 21st day post-treatment; Group I= Diabetic rats without treatment; Group II= Diabetic rats were given glibenclamide; Group III= Diabetic rats with the administration of yogurt + 1% aloe vera extract + 1% fenugreek seed extract; Group IV= Diabetic rats with yogurt + 1% cinnamon extract + 1% aloe vera extract; Group V= Diabetic rats with the administration of yogurt + 1% cinnamon extract + 1% fenugreek seed extract; Group VI= Diabetic rats with yogurt + 1% cinnamon extract + 1% aloe vera extract + 1% fenugreek seed extract.

tween the treatment groups that were given yogurt with the addition of several combinations of herbal extracts (III, IV, V, and VI) showed no significant difference, but group VI showed the lowest blood glucose on day 21, namely 145.20 ± 6.4 compared to the other groups (III, IV, and V).

The results of examining insulin levels in the blood serum of the rats showed that there were significant differences between the untreated group of diabetic rats (I) and the group of treated diabetic rats (II, III, IV, V, and VI) (Table 2.). There was no significant difference between the treatment groups that were given yogurt with the addition of several combinations of herbal extracts (III, IV, V, and VI), but group VI showed the highest insulin levels, namely 1624.92 ± 117.6 compared to the other groups (III, IV, and V).

Lipid Profile (Cholesterol, Triglycerides, HDL, and LDL)

Lipid profiles (cholesterol, triglycerides, and HDL) in experimental rats in this study showed a significant difference between the untreated group of diabetic rats (I) and the treated group (II, III, IV, V, and VI). LDL levels showed significant differences in the untreated group of diabetic rats (I) and the treated group of diabetic rats (II, V, and VI) (Table 3). Still, they showed no significant difference in the treated group (III and IV). In addition, there was no significant difference between the treatment groups that were given yogurt with the addition of several combinations of herbal extracts (III, IV, V, and VI).

Kidney Function

The results of kidney function tests (urea and creatinine) showed that there were significant differences between the untreated group of diabetic rats (I) and the treated groups (II, III, IV, V, and VI). The creatinine content in groups V and VI was not significantly different from the group of diabetic rats that were given glibenclamide but was significantly different from groups III and IV. This was different from the urea content, which was not significantly different between all treatment groups that were given yogurt with the addition of several combinations of herbal extracts (III, IV, V, and VI) and the group of diabetic rats that were given glibenclamide (II) (Table 4).

Liver Function

Tests of liver function in experimental rats in this study showed that there were significant differences in ALT contents between the diabetic rat groups without treatment and the group given glibenclamide (II) and the treatment groups which were given yogurt with the addition of several combinations of herbal extracts (V and VI), but not significantly different from the groups

Table 2. Average insulin and glucose levels in the experimental rat groups

Group	Average fasting blood glucose level (mg/dL)					Average insulin level on day 21 (pg/mL)
	H0	H5	H7	H14	H21	
I	83.20±13.9^{a}	539.20±16.1^{b}	444.80±36.7^{c}	398.20± 9.1^{c}	379.00±25.4^{c}	783.22±171.7^{a}
II	101.20±14.5^{a}	461.40±66.7^{a}	281.60±41.6^{a}	189.00±13.1^{a}	122.60± 5.1^{a}	1786.64±640.7^{b}
III	92.60±19.2^{a}	463.80±31.7^{a}	347.20±11.7^{b}	237.80± 3.7^{b}	156.80± 2.6^{b}	1464.22±796.9^{b}
IV	91.60±12.1^{a}	458.80±25.6^{a}	346.60±13.7^{b}	236.20± 9.9^{b}	156.00± 1.6^{b}	1594.80±279.7^{b}
V	91.20±16.4^{a}	464.00±24.2^{a}	341.20±10.7^{b}	233.40±11.9^{b}	146.20± 4.6^{b}	1581.20±157.2^{b}
VI	88.20± 8.6^{a}	481.40± 6.9^{a}	334.40±10.4^{b}	229.00± 3.1^{b}	145.20± 6.4^{b}	1624.92±117.6^{b}

Note: Means in the same column with different superscripts differ significantly ($p<0.05$). HO= Day before treatment; H5= 5th day post-treatment; H7= 7th day post-treatment; H14= 14th day post-treatment; H21= 21st day post-treatment; Group I= Diabetic rats without treatment; Group II= Diabetic rats were given glibenclamide; Group III= Diabetic rats with the administration of yogurt + 1% aloe vera extract + 1% fenugreek seed extract; Group IV= Diabetic rats with yogurt + 1% cinnamon extract + 1% aloe vera extract; Group V= Diabetic rats with the administration of yogurt + 1% cinnamon extract + 1% fenugreek seed extract; Group VI= Diabetic rats with yogurt + 1% cinnamon extract + 1% aloe vera extract + 1% fenugreek seed extract.

Table 3. The results of the blood lipid profile of the experimental group of rats

Group	Average (mg/dL)			
	Cholesterol	Triglyceride	HDL	LDL
I	99.80±11.50^{c}	119.20± 5.94^{c}	26.60±1.95^{a}	27.00± 3.54^{b}
II	67.30± 3.90^{a}	80.80± 2.77^{a}	38.20±3.03^{c}	20.20± 1.64^{a}
III	76.60± 4.51^{b}	97.70± 8.46^{b}	33.40±3.65^{b}	24.20± 1.48^{ab}
IV	75.60± 3.97^{ab}	95.20±15.55^{ab}	34.20±3.19^{bc}	23.40± 2.30^{ab}
V	73.40± 4.62^{ab}	94.00±12.35^{ab}	36.80±1.92^{bc}	20.20±10.11^{a}
VI	71.80± 3.35^{ab}	93.60±15.89^{ab}	37.40±3.29^{bc}	19.40± 1.67^{a}

Note: Means in the same column with different superscripts differ significantly ($p<0.05$). Group I= Diabetic rats without treatment; Group II= Diabetic rats were given glibenclamide; Group III= Diabetic rats with the administration of yogurt + 1% aloe vera extract + 1% fenugreek seed extract; Group IV= Diabetic rats with yogurt + 1% cinnamon extract + 1% aloe vera extract; Group V= Diabetic rats with the administration of yogurt + 1% cinnamon extract + 1% fenugreek seed extract; Group VI= Diabetic rats with yogurt + 1% cinnamon extract + 1% aloe vera extract + 1% fenugreek seed extract. HDL= high-density lipoprotein cholesterol; LDL= low-density lipoprotein-cholesterol.

Table 4. Results of kidney function tests in experimental group of rats

Group	Average (mg/dL)	
	Urea content	Creatinine content
I	34.32±1.84[b]	0.91±0.27[c]
II	27.84±1.05[a]	0.60±0.05[a]
III	30.72±4.57[a]	0.80±0.05[b]
IV	29.24±3.47[a]	0.79±0.72[b]
V	27.98±0.56[a]	0.64±0.03[a]
VI	27.88±0.58[a]	0.60±0.06[a]

Note: Means in the same column with different superscripts differ significantly (p<0.05). Group I= Diabetic rats without treatment; Group II= Diabetic rats were given glibenclamide; Group III= Diabetic rats with the administration of yogurt + 1% aloe vera extract + 1% fenugreek seed extract; Group IV= Diabetic rats with yogurt + 1% cinnamon extract + 1% aloe vera extract; Group V= Diabetic rats with the administration of yogurt + 1% cinnamon extract + 1% fenugreek seed extract; Group VI= Diabetic rats with yogurt + 1% cinnamon extract + 1% aloe vera extract + 1% fenugreek seed extract.

Table 5. Results of liver function tests in experimental group of rats

Group	Average (U/L)	
	AST Content	ALT Content
I	123.06± 7.50[a]	67.92±67[b]
II	119.64±10.85[a]	57.78± 2.32[a]
III	122.84± 2.77[a]	64.78± 2.41[ab]
IV	120.90± 1.24[a]	63.52± 6.42[ab]
V	119.86± 9.77[a]	58.08± 3.51[a]
VI	118.34± 4.03[a]	56.48± 1.63[a]

Note: Means in the same column with different superscripts differ significantly (p<0.05). Group I= Diabetic rats without treatment; Group II= Diabetic rats were given glibenclamide; Group III= Diabetic rats with the administration of yogurt + 1% aloe vera extract + 1% fenugreek seed extract; Group IV= Diabetic rats with yogurt + 1% cinnamon extract + 1% aloe vera extract; Group V= Diabetic rats with the administration of yogurt + 1% cinnamon extract + 1% fenugreek seed extract; Group VI= Diabetic rats with yogurt + 1% cinnamon extract + 1% aloe vera extract + 1% fenugreek seed extract. AST= aspartate aminotransferase; ALT= alanine aminotransferase.

given yogurt with the addition of several combinations of herbal extracts (III and IV). Different things were shown from testing the AST content, which showed no significant difference between the groups of untreated diabetic rats and all groups of treated rats (II, III, IV, V, and VI). The results of liver function tests can be seen in full in Table 5.

DISCUSSION

Administration of streptozotocin (STZ) in experimental rats can induce diabetes in rats because it is toxic to β-pancreatic cells (Szkuldeski *et al.,* 2013), which is characterized by high glucose levels. Diabetes mellitus is a degenerative disease characterized by hyperglycemia conditions caused by insulin deficiency, insulin resistance, or both and induces dysfunction and failure of various organs. Hyperglycemia conditions can trigger the formation of excess reactive oxygen species (ROS) free radicals in cells and stimulate oxidative stress conditions in the cells (Wresdiyati *et al.,* 2016; Yaribeygi *et al.,* 2019), which trigger insulin resistance and β-pancreatic cell dysfunction (Keane *et al.,* 2015; Hurrle & Hsu, 2017; Barazzoni *et al.,* 2018). In addition to impaired glucose metabolism, people with diabetes mellitus experience impaired protein and fat metabolism, resulting from impaired enzyme work and β-pancreatic damage (Kang & Yang, 2018). This condition was shown in diabetic rats without treatment in this study (I), including high blood glucose levels, low insulin levels, high levels of cholesterol, triglycerides, LDL, urea, creatinine, ALT, and AST, and low levels of HDL (Tables 2, 3, 4, and 5).

Diabetic rats given yogurt with the addition of several combinations of herbal extracts such as cinnamon, aloe vera, and fenugreek seeds (III, IV, V, and IV) and given the commercial drug glibenclamide (II) significantly increased body weight, while diabetic rats without treatment (I) showed significant weight loss. This result is in line with previous studies, which showed that rats induced by STZ and alloxan could lose weight significantly (Wresdiyati *et al.,* 2015). Several previous studies also reported the same thing, that yogurt added with 7% olive leaves herbal extract (Amnah & Alsuhaibani, 2016) and 1.5% olive pomace (Mohamed *et al.,* 2017) was significant in increasing the body weight of diabetic rats.

Yogurt added with some combination of herbal extracts such as cinnamon, aloe vera, and fenugreek seeds (III, IV, V, and VI) can significantly lower blood glucose levels and increase insulin levels in the blood serum of diabetic rats as well as with commercial administration of glibenclamide (II). However, it did not show normal glucose levels until the 21st day post-treatment. The group of diabetic rats that were given glibenclamide decreased faster and achieved normal glucose levels at 21 days post-treatment (Table 2). Blood glucose levels of normal rats ranged from 50-135 mg/dL (Wolfensohn & Lloyd, 2013), and serum insulin levels in normal rats were in the range of 800-1200 pg/mL, while in diabetic rats in the range of 400-700 pg/mL (Wu *et al.,* 2020). Previous studies have reported that giving yogurt and cinnamon soyghurt (*C. burmanii*) can lower blood glucose levels and increase insulin levels in pre-metabolic syndrome rats that experience hyperglycemia (Rustanti *et al.,* 2019). Likewise, research on yogurt with the addition of several herbal extracts such as olive leaves 7% (Amnah & Alsuhaibani, 2016) and olive pomace 1.5% (Mohamed *et al.,* 2017) were significant in lowering blood glucose and increasing insulin levels in diabetic rats.

Yogurt with the addition of several combinations of herbal extracts such as cinnamon, aloe vera, and fenugreek seeds (III, IV, V, and VI) can improve lipid profiles in diabetic rats, especially groups V and VI which show better performance than other groups (III and IV). Previous research conducted by Mohamed *et al*. (2017) with yogurt added with 1.5% olive pomace herbal extract can significantly improve the lipid profile of diabetic rats.

The concentrations of creatinine and urea in blood plasma are used to determine the glomerular filtration

rate of the kidneys and indicators of nephrotoxicity and impaired renal function. Low clearance of creatinine and urea indicates a decreased ability of the kidneys to filter these waste products from the blood to be excreted in the urine. Increased blood plasma creatinine and urea levels indicate impaired kidney function. Likewise, ALT and AST are enzymes that function to digest proteins in the body that are used as biomarkers for liver damage. High levels of ALT and AST in the blood are indicators of impaired liver function (Pal'chikova *et al.,* 2018). This impaired liver function can be seen in the group of untreated diabetic rats (I) in this study.

Diabetic rats with yogurt added with some combination of herbal extracts such as cinnamon, aloe vera, and fenugreek seeds (III, IV, V, and VI) can improve kidney and liver functions. Groups V and VI were able to improve kidney and liver functions, especially ALT levels, which were better than the other groups (III and IV). Previous research also showed that giving yogurt added with 1.5% olive pomace herbal extract can significantly improve kidney and liver functions in diabetic rats (Mohamed *et al*., 2017).

Yogurt with the addition of several combinations of herbal extracts such as cinnamon, aloe vera, and fenugreek seeds, as much as 1% each, proved to be potentially used as antidiabetic and hypolipidemic. The combination of yogurt with several herbal extracts can add efficacy and value to the benefits of yogurt as a functional drink and diversify food products.

Yogurt contains probiotics in the form of lactic acid bacteria such as *Lactobacillus casei* and *Lactobacillus acidophilus,* which are proven to reduce blood glucose levels and prevent type 2 diabetes mellitus by stimulating insulin release, overcoming diabetic dyslipidemia, inhibiting lipid peroxidation and nitrite formation (Zhang *et al.,* 2016). *Lactobacillus* strains can increase serum glucose, inulin, C-peptide, leptin, glycated hemoglobin, GLP-1 levels, IL-6, and TNF-α inflammation in adipose tissue, as well as the expressions of PPAR-γ and GLUT 4 genes (Hsieh *et al.,* 2013). Probiotics can prevent β-pancreatic cell damage by alleviating oxidative stress in pancreatic cells, which can cause chronic inflammation and apoptosis in β-pancreatic cells. The hypoglycemic effect of probiotics is caused by the presence of lactic acid bacteria in the intestinal epithelium by using glucose so that glucose absorption in the intestine is reduced. In addition, the effects of lactic acid inhibitors cause the production of cytokines, which are responsible for β-pancreatic cell damage (Harisa *et al.,* 2009).

Several previous studies have proven that cinnamon has the potential as an antidiabetic (Kusumaningtyas *et al.,* 2014; Beji *et al.,* 2018; Wihansah *et al.,* 2022) and hypolipidemic (Hamidpour *et al.,* 2015; Beji *et al.,* 2018). This effect is influenced by the content of the main flavonoids, which are antioxidants, namely cinnamic aldehyde, cinnamyl acetate, and eucalyptol, which can increase insulin release, reduce intestinal glucose absorption, increase glycogen synthesis, and activate peroxisome proliferator-activated receptor-γ (PPAR-γ) (Muhammad & Dewettinck, 2017). The polyphenol content in cinnamon regulates glucose metabolism and repairs β-pancreatic cells. The content of cinnamaldehyde can serve as a hypoglycemic agent. It can reduce the workload of the pancreas by improving islet function (Li *et al.,* 2013; Zhu *et al.,* 2017). Cinnamon is also beneficial for improving blood lipid profiles because it significantly reduces lipid concentrations and increases HDL cholesterol in the serum (Hamidpour *et al.,* 2015). This effect is because the content of cinnamaldehyde in cinnamon can increase the activity of lecithin cholesterol acyl transferase (Zhu *et al.,* 2017). The polyphenol content in cinnamon can also affect lipid metabolism. Polyphenols effectively inhibit hepatic lipid peroxidation. This effect is beneficial for health because lipid peroxidation produces several products that trigger cytotoxic and genotoxic effects (Li *et al.,* 2013).

Fenugreek seed extract (*T. foenum-graecum* L) added as much as 1% to yogurt proved to have antidiabetic and hypolipidemic potentials. This effect has also been proven by previous studies (Belguith-Hadriche *et al.,* 2013; Baset *et al.,* 2020). Diosgenin saponins are considered bioactive compounds from fenugreek seeds which are antioxidants that play the most role in antidiabetics (Ota & Ulrih, 2017; Tomcik *et al.,* 2017). This compound can repair β-pancreatic cells, stimulate insulin secretion, increase the rate of mRNA transcription from CCAAT/protein enhancer (C/EBPδ) binding, and activate receptor-γ peroxisome proliferator (PPAR-γ) (Nagulapalli *et al.,* 2017; Kumar *et al.,* 2019). Another component in fenugreek seeds, namely 4-hydroxylisoleucine, is an amino acid that can increase insulin secretion and lower plasma triglyceride levels and total cholesterol. In addition, galactomannan is a carbohydrate that represents 45%-60% of fenugreek seeds and has been shown to block the hydrolysis enzymes of carbohydrates and lipids in the digestive system, resulting in a decrease in postprandial glucose levels (Ota & Ulrih, 2017).

Several studies have proven that aloe vera has potential as an antidiabetic and hypolipidemic (Devaraj *et al.,* 2013; Samaneh *et al.,* 2015). Phenolic compounds, flavonoids, and saponins from aloe vera are responsible for hypoglycemic and hypolipidemic effects that can repair β-pancreatic cells, stimulate insulin secretion, and lower blood plasma lipid levels and liver cholesterol. The presence of phenolic compounds and flavonoids is responsible for capturing the free radical superoxide and peroxyl, thus preventing damage to β-pancreatic cells. Phytosterols contained in aloe vera are not absorbed by the intestine but can bind cholesterol and prevent its absorption, causing hypolipidemic effects (lowering blood lipid levels) (Pothuraju *et al.,* 2016).

CONCLUSION

Yogurt with the addition of several combinations of herbal extracts such as cinnamon, aloe vera, and fenugreek seeds, each as much as 1%, has the potential as an antidiabetic and hypolipidemic. Diabetic rats given yogurt with the addition of a combination of 3 herbal extracts (VI) and a combination of 2 herbal extracts (cinnamon and fenugreek seeds) (V) of 1% each showed better antidiabetic and hypolipidemic performances compared to the other groups (III and IV). The combi-

nation of yogurt with some of these herbal extracts can add to the efficacy and value of the benefits of yogurt as a functional drink.

CONFLICT OF INTEREST

The authors declare that they have no competing interest.

ACKNOWLEDGEMENT

This study was funded by the Center of Agricultural Education, Ministry of Agriculture Republic of Indonesia, through a Strategic Research Grant in 2020, number 243/KPA/I/03/2020.

REFERENCES

Abdul, M., B. Khan, M. J. Hashim, J. K. King, R. D. Govender, H. Mustafa, & A. K. Juma. 2020. Epidemiology of type 2 diabetes-global burden of disease and forecasted trends. J. Epidemiol. Glob. Health 10:107-11. https://doi.org/10.2991/jegh.k.191028.001

Al Kury, L. T., Abdoh, A, K. Ikbariah, B. Sadek, & M. Mahgoub. 2022. *In vitro* and *in vivo* antidiabetic potential of monoterpenoids: An update. Molecules 27:1-29. https://doi.org/10.3390/molecules27010182

American Diabetes Association. 2014. Diagnosis and classification of diabetes mellitus. Diabetes Care 37:S81–S90. https://doi.org/10.2337/dc14-S081

American Diabetes Association. 2018. 4. Lifestyle management: Standards of medical care in diabetes. Diabetes Care 41:S38–S50. https://doi.org/10.2337/dc18-S004

Amnah & M. A. Alsuhaibani. 2016. Physicochemical, organoleptic, and antidiabetic, properties of yoghurt fortified with olive leaves. Middle East Journal Applied Sciences 6:341-348.

Barazzoni, R., C. G. Gortan, M. Ragni, & E. Nisoli. 2018. Insulin resistance in obesity: An overview of fundamental alterations. Eat Weight Disord. 23:149–157. https://doi.org/10.1007/s40519-018-0481-6

Baset, M. E., T. I. Ali, H. Elshamy, A. M. El Sadek, D. G. Sami, M. T. Badawy, S. S. Abou-Zekry, H. H. Heiba, M. K. Saadeldin, & A. Abdellatif. 2020. Anti-diabetic effects of fenugreek (*Trigonella foenum-graecum*): A comparison between oral and intraperitoneal administration - an animal study. Int. J. Funct. Nutr 1:1-9. https://doi.org/10.3892/ijfn.2020.2

Beji, R. S., S. Khemir, W. A. Wannes, K. Ayari, & R. Ksouri. 2018. Antidiabetic, antihyperlipidemic and antioxidant influences of the spice cinnamon (*Cinnamomum zeylanicumon*) in experimental rats. Brazilian Journal Pharmaceutical Sciences 54:e17576 1-8. https://doi.org/10.1590/s2175-97902018000217576

Belguith-Hadriche, O., M. Bouaziz, K. Jamoussi, M. S. J. Simmonds, A. El Feki, & F. Makni-Ayedi. 2013. Comparative study on hypocholesterolemic and antioxidant activities of various extracts of fenugreek seeds. Food Chem. 138:1448–1453. https://doi.org/10.1016/j.foodchem.2012.11.003

Chaudhury, A., C. Duvoor, V. S. R. Dendi, S. Kraleti, A. Chada, R. Ravilla, A. Marco, N. S. Shekhawat, M. T. Montales, & K. Kuriakose. 2017. Clinical review of antidiabetic drugs: Implications for type 2 diabetes mellitus management. Front. Endocrinol. 8:1-12. https://doi.org/10.3389/fendo.2017.00006

Chen, M., Q. Sun, E. Giovannucci, D. Mozaffarian, J. E. Manson, C. W. Willett, & F. B. Hu. 2014. Dairy consumption and risk of type 2 diabetes: 3 cohorts of US adults and an updated meta-analysis. BMC Med. 12:1-14. https://doi.org/10.1186/s12916-014-0215-1

Cho, N. H., J. E. Shaw, S. Karuranga, Y. Huang, J. D. da R. Fernandes, A. W. Ohlrogge, & B. Malanda. 2018. IDF diabetes atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res. Clin. Pract. 138:271-81. https://doi.org/10.1016/j.diabres.2018.02.023

Dabija, A., G. G. Codina, S. Ropciuc, A. Gaˆtlan, & L. Rusu. 2018. Assessment of the antioxidant activity and quality attributes of yogurt enhanced with wild herbs extracts. J. Food Qual. 2018:1-12. https://doi.org/10.1155/2018/5329386

Devaraj, S., Y. Mesfin, A. B. Lidia, J. Ishwarlal, S. Sital, & J. Qi. 2013. Effects of *Aloe vera* supplementation in subjects with prediabetes/metabolic syndrome. Metab. Syndr. Relat. Disord. 11:35-40. https://doi.org/10.1089/met.2012.0066

Hamidpour, R., M. Hamidpour, S. Hamidpour, & M. Shahlari. 2015**.** Cinnamon from the selection of traditional applications to its novel effects on the inhibition of angiogenesis in cancer cells and prevention of alzheimer's disease, and a series of functions such as antioxidant, anticholesterol, antidiabetes, antibacterial, antifungal, nematicidal, acaracidal, and repellent activities. J. Tradit. Complement. Med. 5:66–70. https://doi.org/10.1016/j.jtcme.2014.11.008

Harisa, G. I., E. I. Taha, A. F. Khalil, & M. M Salem. 2009. Oral administration of *Lactobacillus acidophilus* restores nitric oxide level in diabetic rats. Aust. J. Basic Appl. Sci. 3:2963-2969.

Hsieh, F., C. Lee, C. Chai, W. Chen, Y. Lu, & C. Wu. 2013. Oral administration of *Lactobacillus reuteri* GMNL-263 improves insulin resistance and ameliorates hepatic steatosis in high fructose-fed rats. Nutr. Metab. 10:1–14. https://doi.org/10.1186/1743-7075-10-35

Hurrle, S. & W. H. Hsu. 2017. The etiology of oxidative stress in insulin resistance. Biomed. J. 40:257–262. https://doi.org/10.1016/j.bj.2017.06.007

Juniawati, Miskiyah, & A. Kusuma. 2020. Penambahan enkapsulan dalam proses pembuatan yoghurt powder probiotik dengan metode spray drying. Jurnal Penelitian Pascapanen Pertanian 16:56-63. https://doi.org/10.21082/jpasca.v16n2.2019.56-63

Kaminskas, A., J. A. Abaravičius, A. Liutkevičius, V. Jablonskienė, & J. Valiūnienė. 2013. Quality of yoghurt enriched by inulin and its influence on human metabolic syndrome. Veterinarija Ir Zootechnika 64:23-28.

Kang, H. S. & D. K. Yang. 2018. Anti-diabetic effect of cotreatment with quercetin and resveratrol in streptozotocin-induced diabetic rats. Biomol. Ther. 26:130–138. https://doi.org/10.4062/biomolther.2017.254

Keane, K. N., V. F. Cruzat, R. Carlessi, P. I. H. De Bittencourt, & P. Newsholme. 2015. Molecular Events linking oxidative stress and inflammation to insulin resistance and β-cell dysfunction. Oxid Med. Cell Longev. 2015:181643. https://doi.org/10.1155/2015/181643

Kottaisamy, C. P. D., D. S. Raj, P. Kumar, & U. Sankaran. 2021. Experimental animal models for diabetes and its related complications-a review. Lab. Anim. Res. 37:1-14. https://doi.org/10.1186/s42826-021-00101-4

Kumar, A., S. Aswal, A. Chauhan, R. B. Semwal, A. Kumar, & D. K. Semwal. 2019. Ethnomedicinal investigation of medicinal plants of Chakrata region (Uttarakhand) used in the traditional medicine for diabetes by Jaunsari tribe. Nat. Prod. Bioprospect. 9:175-200. https://doi.org/10.1007/s13659-019-0202-5

Kusumaningtyas, I. D., S. Fajariyah, & E. T. Utami. 2014. The effect of cinnamon (*Cinnamomum burmanii*) aqueous extract on pancreas structure of diabetic mice (*Mus musculus*) strain Balb-C. Jurnal Ilmu Dasar 15:69-73. https://doi.

org/10.19184/jid.v15i2.813

Li, R., T. Liang, L. Xu, Y. Li, S. Zhang, & X. Duan. 2013. Protective effect of cinnamon polyphenols against stz diabetic mice fed high-sugar, high-fat diet and its underlying mechanism. Food Chem. Toxicol 51:419–425. https://doi.org/10.1016/j.fct.2012.10.024

Ligita, T., K. Wicking, K. Francis, N. Harvey, & I. Nurjannah. 2019. How people living with diabetes in Indonesia learn about their disease: A grounded theory study. PLoS One 14:1-19. https://doi.org/10.1371/journal.pone.0212019

Ma, W., L. Xiao, H. Liu, & X. Hao. 2022. Hypoglycemic natural products with *in vivo* activities and their mechanisms: a review. Food Science Human Wellness 11:1087-1100. https://doi.org/10.1016/j.fshw.2022.04.001

Mohamed, D. A., M. M. Hassanein, T. M. El-Messery, M. T. Fouad, M. M. El-Said, K. A. Fouda, & A. G. Abdel- Razek. 2017. Amelioration of diabetes in a rat model through yoghurt supplemented with probiotic and olive pomace extract. Int. J. Biol. Sci. 17:320-333. https://doi.org/10.3923/jbs.2017.320.333

Muhammad, D. R. A. & K. Dewettinck. 2017. Cinnamon and its derivatives as potential ingredient in functional food-a review. Int. J. Food Prop. 20:S2237–S2263.

Nagulapalli, V. K. C., A. Swaroop, D. Bagchi, & A. Bishayee. 2017. A small plant with big benefits: Fenugreek (*Trigonella foenum graecum Linn*.) for disease prevention and health promotion. Mol. Nutr. Food Res. 61:1600950. https://doi.org/10.1002/mnfr.201600950

National Research Council. 2011. Guide for The Care and Use of Laboratory Animals. 8th ed. National Academic Press, Washington, DC, US. p. 246.

National Research Council. 1995. Nutrient Requirements of Laboratory Animals. 4th ed. National Academy Press, Washington, DC, US. p. 29-30.

Ota, A. & N. P. Ulrih. 2017. An overview of herbal products and secondary metabolites used for management of type two diabetes. Front. Pharmacol. 8:1-14. https://doi.org/10.3389/fphar.2017.00436

Pal'chikova, N. A., V. G. Selyatitskaya, O. I. Kuz'minova, & K. V. Pasechnaya. 2018. Effects mifepristone on aminotransferase activities in the liver in rats with streptozotocin-induced diabetes mellitus. Bull. Exp. Biol. Med. 165:474–477. https://doi.org/10.1007/s10517-018-4197-4

Pothuraju, R., R. K. Sharma, S. K. Onteru, S. Singh, & S. A. Hussain. 2016. Hypoglycemic and hypolipidemic effects of aloe vera extract preparations: A review. Phytother. Res. 30:200–207. https://doi.org/10.1002/ptr.5532

Rustanti, N., V. Z. Nafsih, R. N. Avisha, D. M. Kurniawati, R. Purwanti, C. Nissa, H. S. Wijayanti, & D. N. Afifah. 2019. Pengaruh yoghurt dan soyghurt kayu manis (*Cinnamomum burmannii*) terhadap kadar glukosa darah, insulin serum, dan malondialdehyde tikus pra sindrom metabolik. Jurnal Gizi Indonesia 8:60-68. https://doi.org/10.14710/jgi.8.1.60-68

Saeedi, P., I. Petersohn, P. Salpea, B. Malanda, S. Karuranga, N. Unwin, S. Colagiuri, L. Guariguata, A. A. Motala, K. Orgutsowa, J. E. Shaw, D. Bright, & R. Williams. 2019. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the international diabetes federation diabetes atlas, 9th edition. Diabetes Res. Clin. Pract. 157:107843. https://doi.org/10.1016/j.diabres.2019.107843

Salehi, P., B. Asghari, M. A. Esmaeili, H. Dehghan, & I. Ghazi. 2013. α -Glucosidase and α -amylase inhibitory effect and antioxidant activity of ten plant extracts traditionally used in Iran for diabetes. Res. J. Medicinal Plant. 7:257–266.

Samaneh, A., F. Mohsen, A. S. Seyed, & S. Majid. 2015. Improvement of glucose and lipid profile status with *Aloe vera* in pre-diabetic subjects: A randomized controlled-trial.J. Diabetes Metab. Disord. 14:1-7. https://doi.org/10.1186/s40200-015-0137-2

Tomcik, K. A., W. J. Smiles, D. M. Camera, H. M. Hügel, J. A. Hawley, & R. Watts. 2017. Fenugreek increases insulin-stimulated creatine content in L6C11 muscle myotubes. Eur. J. Nutr. 56:973-979. https://doi.org/10.1007/s00394-015-1145-1

Westman, E. C. 2021. Type 2 diabetes mellitus: A pathophysiologic perspective. Front. Nutr. 8:1-5. https://doi.org/10.3389/fnut.2021.707371

Wresdiyati, T., S. Sa'diah, & A. Winarto. 2016. The antidiabetic properties of Indonesian *Swietenia mahagoni* in alloxan induced-diabetic-rats. World Academy Science, Engineering, Technology International Journal Animal Veterinary Sciences 10:631–637.

Wresdiyati, T., S. Sa'diah, A. Winarto, & V. Febriyani. 2015. Alpha-glucosidase inhibition and hypoglycemic activities of *Sweitenia mahagoni* seed extract. HAYATI J. Biosci. 22:73–78. https://doi.org/10.4308/hjb.22.2.73

Wolfensohn, S. & M. Lloyd. 2013. Handbook of Laboratory Animal Management and Welfare. 8th ed. Blackwell Science Ltd, Oxford (UK).

Wu, G., Z. Bai, Y. Wan, H. Shi, X. Huang, & S. Nie. 2020. Antidiabetic effects of polysaccharide from azuki bean (*Vigna angularis*) in type 2 diabetic rats via insulin/PI3K/AKT signaling pathway. Food Hydrocoll. 101:105456. https://doi.org/10.1016/j.foodhyd.2019.105456

Yaribeygi, H., S. L. Atkin, & A. Sahebkar. 2019. A review of the molecular mechanisms of hyperglycemia-induced free radical generation leading to oxidative stress. J. Cell Physiol. 234:1300–1312. https://doi.org/10.1002/jcp.27164

Zeng, Z., J. Luo, F. Zuo, Y. Zhang, H. Ma, & S. Chen. 2016. Screening for potential novel probiotic *Lactobacillus* strains based on high dipeptidyl peptidase IV and α-glucosidase inhibitory activity. J. Funct. Foods. 20:486-495. https://doi.org/10.1016/j.jff.2015.11.030

Zhang, Q., Y. Wu, & X. Fei. 2016. Science direct effect of probiotics on glucose metabolismin patients with type 2 diabetes mellitus: A meta-analysis of randomized controlled trials. Medicina (B. Aires) 52:28–34. https://doi.org/10.1016/j.medici.2015.11.008

Zhu, A. R., H. Liu, C. Liu, L. Wang, R. Ma, B. Chen, L. Li, J. Niu, M. Fu, D. Zhang, & S. Gao. 2017. Cinnamaldehyde in diabetes : A review of pharmacology, pharmacokinetics and safety. Pharmacol. Res. 122:78-89. https://doi.org/10.1016/j.phrs.2017.05.019