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RESEARCH ARTICLE

Effects of a combination of *Sauropus androgynus L.* leaf and *Zingiber Ottensii* rhizome on fatty acid profile and liver damage in rats

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Keywords
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Abstract

Background: High fat and carbohydrate diets may lead to the malfunction of hepatic cells because of excessive lipolysis of fat tissues and the subsequent increase of free fatty acids in the liver. This is characterised by histological changes in the hepatic cells which then undergo necrosis. High fat and carbohydrate diets may also lead to gut microbiota *dysbiosis.* **Objectives:** This study aims to determine the effect of the combination of *katuk* leaf and black bangle rhizome on the short chain fatty acid (SCFA) profile and liver cell damage in rats induced by a high fat and carbohydrate diet. **Methods:** The research method used is a preventive experimental study *in vivo*. Four groups of test animals were distinguished into negative group, positive group, comparative group, and 15% combination test group, which was carried out for 21 days. Examined parameters were: SGPT (Serum Glutamic Pyruvic Transaminase), SGOT (Serum Glutamic Oxaloacetic Transaminase), Triglyceride, and hepatic cells histopathology. The results were examined by SPSS and showed a significant difference ($p < 0.05$). **Results**: Parameter results showed the reduction of SGOT in the combination test group. The combination of *katuk* leaf and black bangle rhizome can increase acetic acid and lower the *Manja Roenigk* score, and it is possible to inhibit liver cell damage. **Conclusion:** The combination of *katuk* leaf (*Sauropusxandrogynus* L.Merr) and black bangle rhizome (*Zingiber ottensii* Val) may affect the SCFA levels and help lower the risk of liver cell damage.

Introduction

A poor diet with high-fat and high-carbohydrate foods has an impact on people health, one of which is obesity (Cerdó *et al*., 2019). Obesity rates in Indonesia are on the rise; the population in the overweight category for those over 18 years old has increased from 8.6% in 2007 to 11.5% in 2013 and 13.6% in 2018 (Riskesdas, 2018). For the whole Indonesian population, the percentage in the obesity category was 10.5% in 2007 and rose to 14.8% in 2013 and 21.8% in 2018 (Riskesdas, 2018).

The pathophysiology of obesity starts from an imbalance of calories taken in and burnt off; sedentary lifestyles are known to cause fat accumulation. The excess fat may lead to a change in inflammatory homeostasis and the escalation of blood lipid concentration (Kim *et al.*, 2018). Adipose tissue acts as an energy store and endocrine organ; it can be divided into White Adipose Tissue (WAT) and Brown Adipose Tissue (BAT) by their ability to oxidise acids, fat, glucose and maintain body temperature (Baqai, 2014). In people with obesity, it may cause insulin resistance, dyslipidemia, NAFLD (Non-Alcoholic Fatty Liver Disease), and metabolic syndrome (Jung & Choi, 2014).

NAFLD begins from NASH (Nonalcoholic steatohepatitis) without any history of significant alcohol intake (Belletani, 2017). The liver disorder may lead to fat accumulation and cause it to malfunction. It is likely to occur in obese people. Saturated fatty acid oxidation supplies the other organ functions, and the beta

oxidised fatty acid forms Acetyl Coenzyme-A (Sakamoto *et al*., 2017). The studies on obesity were focused on excessive and unhealthy food intake and lack of physical activity; however, they also suggested that gut microbiota composition may be linked to other metabolic syndrome diseases, including diabetes mellitus type 2 and hyperlipidemia (Blaut, 2015).

The gut microbiota is composed of more than 100 trillion cells of complex microorganisms, and approximately 400 species live in a human host bowel. It catabolises fibre from dietary intake and is partially broken down by colonic enzymes. It also ferments short chain fatty acids (SCFAs) into acetic acid, propionic acid and butyric acid (Venegas *et al*., 2019). Studies have also shown the ability of gut microbiota to decrease the inflammatory reaction for such metabolic disorders, such as insulin resistance, dyslipidemia and fatty liver (Carrera-Quintanar *et al*., 2018). SCFA synthesises lipids and glucose.

Indonesia has various plant species such as *Katuk* leaf (*Sauropus androgynus* L. Merr) and black bangle rhizome (*Zingiber ottensii Val*.), which are widely used to treat various diseases (Patonah Susilawati, & Riduan, 2017). A previous study by Sulaeman and Negara (2018) mentions the active compounds of both plants had proven to be effective in preventing weight gain in rats on a high-fat, high-carbohydrate diet. It is on this backdrop that further study on the effectivity of *Katuk* leaf (*Sauropus androgynus* L. Merr*)* and black bangle rhizome (*Zingiber ottensii Val*.) on SFCA profile and liver histopathology in obese male Wistar rats was performed. The results from this study are expected to contribute to discussions on the use of *Katuk* leaves and Black bangle rhizomes as preventive medicines for liver damage by observing the SCFA profile and liver damage of rats with high fat and high carbohydrate intake.

Method

Simplicia collection

Simplicia collection of the black bangle (*Zingiber ottensii Val*) and *Katuk* leaf (*Sauropus androgynus* L.Merr) was obtained from BALITRO (Research Institute for Spices and Medicinal Plants), Jalan Tentara Pelajar No.3, Menteng Bogor, Indonesia**.**

Plant determination

Plant determination was carried out in the Plant Taxonomy Laboratory of Padjadjaran University Faculty of Mathematics and Science.

Feed composition

The composition of normal and high-fat, high-carbohydrate diets is shown in Table I. Preparation of test feed or Simplicia feed consisted of high-fat and carbohydrate-rich induction feed mixed with Simplicia of *Katuk* leaf (*Sauropus androgynus* L. Merr) and black *bangle* (*Zingiber ottensii Val*).

Table I: Composition of normal feed and high-fat high-carbohydrate feed

No	Ingredient	Normal feed (%)	High-fat high-carbohydrate feed (%)
1	Corn flour	25	25
2	Fish flour	16	16
3	Mung bean flour	14	14
4	Flour	41	13
5	Vegetable oil	4	
6	Beef oil		32
	Total	**100**	**100**

Source: (Patonah Susilawati, & Riduan, 2017)

Preparation and treatment of test animals

Male Wistar rats weighing 180-250 grams between two to three months old were used. The Padjadjaran University Health Research Ethics Commission approved this study. The rats were housed in a suitable cage for seven days before the study and were fed and watered as standard. The 20 rats were split into four groups. Each group had five rats. The negative group was fed standard feed and 0.5 % sodium CMC. High fat, high carbohydrate diets and 5% sodium CMC induced positive group rats. High fat, high carbohydrate, and curcumin (0.360mg/200g body weight in 0.5% Sodium CMC suspension) induced the comparative group rats. High fat, high carbohydrate, and Simplicia combination of *Katuk* leaf and black *bangle* (7.5%; 7.5%) and 0.5% sodium CMC were induced in 15% Combination test group rats. Except for the normal group, all groups received a 25% fructose solution. Animals were weighed daily. Triglycerides, SGOT and SGPT enzymes, and liver histology were assessed after day 21.

Biochemistry parameters examination

Analysis of SGPT (Serum Glutamic Pyruvic Transaminase), SGOT (Serum Glutamic Oxaloacetic Transaminase), and triglycerides was performed using a Microlab 300 Photometer.

SCFA examination

SCFA examination was performed by GCMS (Gas Chromatography-Mass Spectrometry). Calibration curve of SCFA consisted of SCFA standard stock

(standard mix: acetic, formic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic, caproic dan heptanoic) with ten mmol concentration and internal standard stock of SCFA (96%) 2,2-Dimethyl butyric acid with a concentration of 921 mg/ml. It was diluted by eight levels of dilution with a concentration rate of 0.670 – 0.005 mmol.

Faeces examination for SCFA

Examined faeces taken in T21 (day 21) were crushed and weighed. A suspension of faeces was made from diluent consisting of 980µl distilled water and 20µl internal standard sample solution; therefore, the total volume was 1000µl, and it was then homogenised by a vortex. The first sonication step was performed for 20 minutes and centrifuged for five minutes with 10,000rpm speed. The supernatant was taken for the following step preparation. 100µl supernatant was taken and diluted with 300µl distilled water, a vortex homogenised a total volume of 400µl. Approximately 100µl supernatant was taken and entered to GC bottle, diluted with 75µl diluent two consisted of 425µl and HCL 1.5N and total volume of both mixes are 600µl and homogenised by the vortex.

Liver histopathological examination

Histopathological examination of liver tissue of the rats was carried out in General Ahmad Yani University, Bandung. One liver tissue was taken from each group of tests. Liver histology staining was done using hematoxylineocyn, the damaged hepatic cells of rats were documented and observed. Histological results were analysed using the Manja Roenigk modification method to determine the store of liver histology based on the damage. The best data selected each group, and each microscope slide would have five selected vision spaces, and each space has 20 observed hepatic cells.

Data processing

Statistical data analysis was performed using SPSS with one way ANOVA method to verify the significance of the average difference between groups by looking at the difference in treatment between groups. All data obtained were compared statistically with a probability value of $p \leq 0.05$.

Ethical exemption of the research

The animals used in this study were male Wistar rats. The use of the rats has been approved by the Research Ethics Commission of the University of Padjadjaran Bandung, which is written in the Ethics Exemption Letter Number of 176/UN6.KEP/EC/2021.

Results

Phytochemical screening

Phytochemical screening on Simplicia of *Katuk* leaf (*Sauropus androgynus* L.Merr) and black bangle rhizome (*Zingiber ottensii Val*) can be seen in Table II.

Table II: Simplicia phytochemical screening results

Compound group	Black bangle rhizome Results of test	Black bangle rhizome (Patonah Susilawati, & Riduan, 2017)	*Katuk* Leaf Results of test	*Katuk* Leaf (Susanti, Budiman, & Warditiani, 2015)
Alkaloids	+	-	+	+
Flavonoids	+	+	+	+
Saponins	+	+	+	+
Steroids	+	-	+	-
Triterpenoids	+	+	+	+
Quinone s	-	+	-	+
Tannins	+	-	+	+

(+) Positive detection of secondary metabolites
(-) Negative detection of secondary metabolites

Measurement of biochemical parameters

Measurement of biochemical parameters can be seen in Table III.

Table III: Average level of T21 biochemical parameters in each group

Group	SGPT (IU) (Mean±SD)	SGOT (IU) (Mean±SD)	Triglyceride (mg/dl) (Mean±SD)
Negative	26.00 ± 4.00	103.77 ± 12.66	167.00 ± 6.55
Positive	44.67 ± 2.88	216.00 ± 9.84	109.67 ± 8.08
Comparative	49.67 ± 7.50	133.00 ± 49.87	126.00 ± 6.00[b]
Combination test	64.33 ± 7.02[a]	140.50 ± 17.50[a]	135.00 ± 22.06[a]

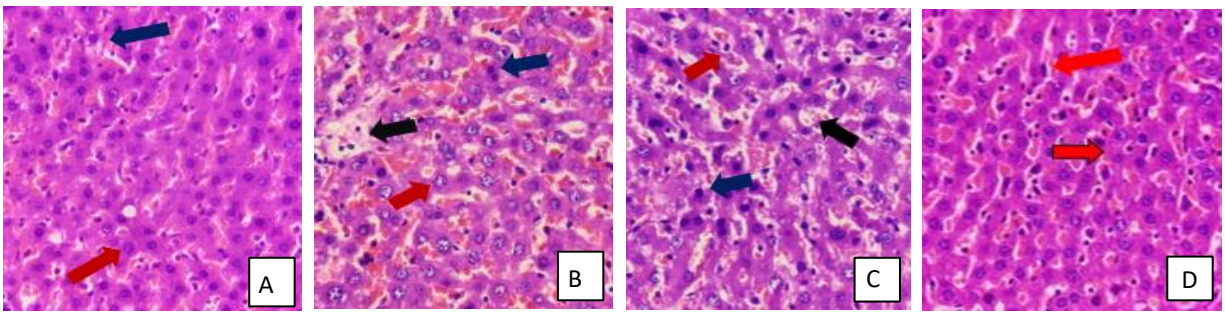
a : significantly different when compared to the positive group
b : significantly different when compared to the comparative group

Effect of the combination of Black Bangle and Katuk Leaf on liver histopathology

The histopathological results can be seen in Figure 1.

Manja Roenigk scoring profile and SCFA profile

Manja Roenigk Scoring Profile and SCFA profile can be seen in Table IV.



Note: Red arrow (Normal cells); Blue arrow (Hydrophilic degenerative cells); Black arrow (Necrotic cells)
a) Negative group: negative group or normal cells are pointed by the red arrow, hydrophilic degenerative cells inside cytoplasm cell are pointed by the blue arrow; **b) Positive group**: dead cells were seen with characteristics of the shrinking cell nucleus as darker spot (pictonic) spots pointed by the blue arrow. Black arrow pointed dead cells, and there were only a few normal cells pointed by the red arrow as chromatin; **c) Comparative group**: dark spots (pictonic) spots pointed by the blue arrow are dead cells. There was also cytoplasm without a nucleus (karyolysis) pointed by the black arrow. The red arrow shows normal cells containing chromatin; **d) Combination Test Group**: There are red spots as the nucleus pointed by red arrows.

Figure 1: Liver histopathology in test animals with 400x magnification

Table IV: *Manja Roenigk* scoring and SCFA profile

Group	Histopathology Mean±SD	SCFA Level (mg/mL)		
		Acetate	Butyrate	Propionate
Negative	1.10 ± 0.10	42.24	30.39	6.49
Positive	2.32 ± 0.42	39.26	16.36	7.98
Comparative	2.08 ± 0.39	53.22	-	-
Combination test	1.16 ± 0.15	47.98	13.57	1.06

Discussion

Phytochemical screening shows there are alkaloids, flavonoids, saponins, triterpenoids, quinones, and tannins in *Katuk* leaf; meanwhile, black bangle rhizome shows the existence of alkaloids, flavonoids, saponins, steroid/triterpenoids, and tannins. The study used a preventive approach, with rats induced and treated for 21 days. The combination of *Katuk* leaf *(Sauropus androgynus* L.Merr) and black bangle rhizome (*Zingiber ottensii Val*) compared to curcumin in preventing body weight gain in test animals. A previous study by Sulaeman & Negara (2018) shows that a high-fat, high-carbohydrate diet leads to the escalation of deposited fat in adipose tissue. After 21 days of inductions, the bodyweight of the test animal was not 20% of its initial weight. Longer feeding intervals cause this. A high-fat high-carbohydrate diet was given for 42 days to achieve a 20% increase in body weight. After 21 days of inductions, the bodyweight of the test animal was less than 20% of its initial weight. Longer feeding intervals could be responsible for this. A high fat, high carbohydrate diet was given for 42 days to achieve a 20% increase in body weight (Yuniarto, Kurnia, & Ramadhan, 2015).

The combined weight of black bangle rhizome (*Zingiber ottensii Val*) and *Katuk* leaf (*Sauropus androgynus* L.Merr) fed rats decreased significantly after 21 days of induction. Weight gain as a result of high-fat high-carbohydrate diets can be reduced by a combination of black bangle rhizome and *Katuk* leaf (*Zingiber ottensii Val*) in test animals. On the 14th day, the rats gained weight on average in the comparative group. Curcumin's pharmacological effects include hepatoprotection, anti-inflammatory, and appetite stimulation.

A high-fat high-carbohydrate diet may cause liver dysfunction because the liver regulates fat metabolism. Hepatic enzymes are released into the blood when hepatic cells are damaged. The results show that the Combination Test and Positive groups have different SGPT, SGOT, and Triglyceride levels. The negative and positive groups did not differ significantly. The 21-day high-fat high-carbohydrate induction had no effect on positive group parameters, and there was no fat accumulation in the liver. A previous study (Patonah Susilawati & Riduan, 2017) found that a high fat, high carbohydrate diet for 21 days increased the triglycerides of test animals.

Triglyceride buildup in the liver can cause hepatocyte necrosis. An increase in oxidative stress can cause hepatocyte inflammation. This occurs in mitochondria and may lead to cell death. Transaminase enzyme levels in hepatocytes detect hepatic cell damage. SGOT and SGPT levels would rise. The permeability of damaged or dead hepatocytes is different to that of healthy cells, causing an increase in SGOT and SGPT levels in the blood.

The positive group had more necrotic cells than the negative group after 21 days of a high-fat high-carbohydrate diet. The findings show that a high-fat high-carb diet can harm the liver. The lively group has a

higher average score than other groups, indicating necrotic cells in the positive group. A higher score indicates poor liver health, as represented by the positive group. The combination group's results show no necrotic cells. The results from both the negative and positive groups showed that the nuclei were intact, which shows that the black *bangle* (*Zingiber ottensii Val*) and *Katuk* leaf (*Sauropus androgynus L.Merr*) may protect hepatic cells and organs from damage. Unlike the control group, curcumin had pyknosis cells and some necrosis. Contrary to the findings from a study by Marinda (2014), curcumin can prevent liver damage in rats caused by high-fat and high-carbohydrate diets. Changes in the cytoplasm and nucleus of hepatic cells indicate necrosis (Takukude *et al.,* 2014). The morphology of the cell nucleus (pyknosis) changes first (Adikara *et al*., 2013). The next stage is nucleus rupture and karyolysis. Pyknosis occurs when cells lose their ability to eliminate water and triglycerides, causing water and triglycerides to accumulate in the cytoplasm. Strong toxins metabolic disorders (fat, protein, and viral infections) can cause necrosis (Swarayana *et al*., 2017).

Induction with high-fat and high-carbohydrate can cause obesity. Dietary intake can be modified to reduce the risk of obesity by promoting the consumption of healthy foods, such as dietary fibre. Dietary fibre is a non-digestible plant cell wall residue that can improve digestion, prevent colon cancer, lower blood glucose, act as a prebiotic, control obesity, and lower blood fat levels.

The *Katuk* leaf contains about 1% dietary fibre, while the black bangle rhizome contains 6% dietary fibre and 48% starch (Evizal, 2013; Santoso, 2016). These are weak organic acids with 1-6 carbon ions produced by the colon and distal small intestine microbiota through carbohydrate fermentation (Fairudz, 2015). There was acetic acid (C2), butyric acid (C3) and propionic acid (C4) in over 95% of the SFCA produced. The colonic lumen SCFA concentration ratio is 60% acetate, 25% propionate, and 15% butyrate. The number and strains of bacteria in gut microbiota, substrate sources, and intestinal transit time all influence SCFA content in the colon.

The test group had a more extensive SCFA profile than the positive group. Simplex *Katuk* leaf (*Sauropus androgynus L. Merr*) combined with black bangle rhizome (*Zingiber ottensii Val*) for 21 days affects intestinal metabolism microbiota producing SCFA. Compared to the positive group, it had a lower SCFA profile. SCFA formation can inhibit cholesterol synthesis and triglyceride secretion, potentially lowering cholesterol capacity (Justyn, 2019).

The curcumin group had a higher acetate profile with low butyrate and propionate levels. In the curcumin comparison group, propionic acid and butyric acid were not detected in the percentage of the profile obtained. This is because some faeces are not completely dry, and some are not mixed homogeneously during sample preparation.

Propionate inhibits the synthesis of d-cholesterol, while butyrate is thought to promote cancer growth (Brownlee, 2016). Acetic acid promotes lipid metabolism via the FFAR2 receptor in WAT, while propionic acid promotes NAFLD by increasing lipogenesis in the liver, decreasing insulin sensitivity, and decreasing fatty acid oxidation (Juárez-Hernández *et al*., 2016). FFAR2 is an SCFA receptor activated by acetic and propionic acid. FFAR2 is found in adipose, digestive, and immune cells. These receptors promote the release of GLP-10 (Glucagon-Like Peptide-1) and PYY (Peptide YY). GLP-1 release in adipose cells can reduce fat accumulation (Nilsson *et al*., 2003). Butyric acid, produced by SCFA, has been shown to reduce lipid deposition in the liver, insulin resistance, and inflammation (Justyn, 2019). In a combination test group of *Katuk* leaf and black bangle rhizome, SGOT decreased compared to the positive group, as shown by the SCFA profile. The combination of *Katuk* leaf and black bangle rhizome prevented hepatic cell damage in the test group.

Study limitation

Limitations of this study are the 21-day induction time and SCFA measurements in the sample of the study.

Conclusion

The combination of *Katuk* leaf (*Sauro-pus androgynus* L.Merr) and black bangle rhizome (*Zingiber ottensii Val*) affects the SCFA level, thereby may decrease the risks of liver damage.

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