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Research article

Blood pressure- lowering and anti-obesity potential of sacha inchi tempeh (*plukenetia volubilisl.*) : Study in metabolic syndrome rats

Muhammad Dzulkha Ikhsanul Fikri[1], Amalia Rani Setyawati[1], Nafisah[1], Gemala Anjani[1,2], Ahmad Syauqy[1,2], Endang Mahati[3], Made Astawan[4], Irma Sarita Rahmawati[5], Irwandi Jaswir[1,6,7], Diana Nur Afifah*[1,2]

[1] Department of Nutrition Science, Faculty of Medicine, Diponegoro University, Semarang, Indonesia
[2] Center of Nutrition Research (CENURE), Diponegoro University, Semarang, Indonesia
[3] Department of Pharmacology, Faculty of Medicine, Diponegoro University, Semarang, Indonesia
[4] Department of Food Science and Technology, IPB University, Bogor, Indonesia
[5] Department of Nutrition, Faculty of Medicine, Brawijaya University, Malang, Indonesia
[6] Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Negeri Padang, Indonesia
[7] International Institute for Halal Research and Training, International Islamic University Malaysia, Kuala Lumpur, Malaysia

Corresponding author: Diana Nur Afifah, ✉ nurafifah.dna@fk.undip.ac.id, **Orcid Id**: https://orcid.org/0000-0001-08808-1826
Department of Nutrition Science, Faculty of Medicine, Diponegoro University, Semarang, Indonesia

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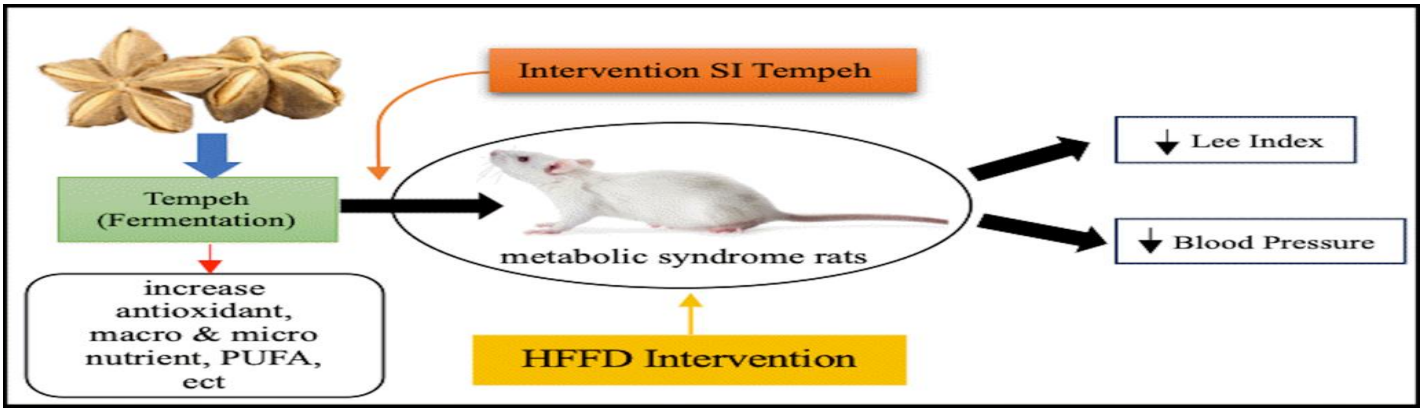
ABSTRACT

Metabolic syndrome is an error in metabolism characterized by dyslipidemia, insulin resistance, hypertension, and central obesity which is a risk factor for cardiovascular disease and stroke. Prevention of metabolic syndrome is by consuming foods that contain high levels of bioactive compounds, for example, sacha inchi. Tempeh is a fermented food native to Indonesia which can increase the content of antioxidants and phenolic compounds which can help cure metabolic syndrome and hypertension. This is an experimental study with a pre-test and post-test with a control group as the design with the independent variable sacha inchi tempeh and the dependent variables blood pressure and Lee index. The number of rats samples was 36 and divided into 6 groups, namely K0 as healthy control, K- as diseased control, K+ (Simvastatin 0.18 g), P1 (0.9 dose), P2 (1.8 dose) and P3 (dose 3.6) and given intervention for 35 days.



Data analysis used the statistical application of the Paired T-test and one-way ANOVA test, followed by the LSD post-hoc analysis. The results obtained by blood pressure and Lee index were P=<0.005 and the ANOVA results showed significant results (P=<0.05) in each group with the most effective treatment being P2. Conclusion: Sacha Inchi tempeh has an effect in improving b in metabolic syndrome rats, blood pressure, and Lee index with P2 as the most effective treatment and the effect is the same as medication so it can be used as an alternative functional food for metabolic syndrome individuals.

Keywords: Blood Pressure, Obesity, Lee Index, Metabolic Syndrome, Sacha Inchi, Tempeh

INTRODUCTION

The metabolic disease known as metabolic syndrome is characterized by dyslipidemia, insulin resistance, hypertension, and central obesity [1]. Metabolic syndrome is a risk factor for the development of coronary heart disease and stroke, which are the largest causes of death in the world [2]. A meta-analysis shows that the prevalence of metabolic syndrome in the world is currently 31.4% and continues to increase [3]. Symptoms of metabolic syndrome are hypertension which is characterized by blood pressure >130 mmHg and central obesity which is characterized by BMI >30 kg/m3 or waist ratio >0.9 (men) and >0.85 (women) [2]. The cause of the metabolic syndrome is a diet that is high in saturated fat, high in carbohydrates, and low in fiber followed by a sedentary lifestyle [4].

Metabolic syndrome can be prevented by consuming foods containing antioxidants, fiber, and other bioactive compounds which can have an ameliorative effect on metabolic syndrome [5]. Sacha inchi (SI) is a seed originating from the Amazon and becoming a functional food. It contains antioxidant compounds such as Polyunsaturated Fatty Acid (PUFA) as Linoleic acid (LA), Linolenic acid (ALA), various vitamins and minerals which in various studies have beneficial effects. which is good for health [6–10]. In research conducted by Yi En et al. (2024), there was an improvement in blood pressure after administering 1.06 grams of sacha inchi oil for 2 months [7]. This anti-hypertensive effect is because the ALA contained in sacha inchi inhibits the synthesis of TxA2 (thromboxane A2) which is a vasoconstrictor and is activated when inflammation occurs which causes blood pressure to decrease [11]. Various studies also show the effects of PUFA contained in Sacha inchi can improve obesity in both experimental animals and humans.[12] This weight loss effect is because ALA can reduce the hormone leptin which reduces appetite decreases triglyceride production and prevents the accumulation of visceral fat [12].

Tempeh is a fermented product native to Indonesia [13]. Various studies can increase the content of antioxidants, and polyphenols, break down proteins [14–16]. Fermentation can remove anti-nutritional compounds such as tannins and saponins [17]. This increase in bioactive and antioxidant content occurs due to the activity of microorganisms such as Rhizopus which causes anaerobic metabolic reactions and the emergence of enzymes such as fibrinolytics and proteases [18,19]. Currently, soybeans are the main ingredient and are most often used as fermented products, and sacha inchi tempeh has never been made and its effect in improving metabolic syndrome is not yet known [17].

This research aims to look at the potential of sacha inchi tempeh for blood pressure and improving obesity and has never been studied before, considering that sacha inchi has bioactive compounds that can improve the condition of metabolic syndrome.

METHODOLOGY

The ingredients used are Sacha Inchi nuts (*Plukenetia volubilis* L.) (Sari Bhumi®), *Rhizopus oligosporus* yeast (Raprima®), Standard Rat Feed CP 594 (Comfeed), High Fat Fructose Diet (HFFD) in the form of pork oil (20%), Cholesterol (1.5%), folic acid (0.5%) and fructose as much as 1 ml/ 200g/bb, Simvastatin 0.18 g (Kimia Farma®), Wistar rats age 8 - 10 weeks and body weight before treatment ± 150 - 200 grams were obtained from the Laboratory of the Center for Food and Nutrition Studies, Gadjah Mada University, banana leaves and rubber.

The tools used are a tray (Lionstar), a steamer (Maspion), a digital scale (SF-400Ò), a pan (Maspion), a stainless steel basin (Maspion), a thermometer (Pyrex), an incubator (B-One, China), a stove (Rinai), sonde (Promedika), micropipette (Dragonlab), injection syringe (Terumo, Japan), Sphygmomanometer.

Preparation of Sacha Inchi Tempeh

500 g of Sacha Inchi seeds were washed with distilled water, then soaked in distilled water for 1 hour, then boiled at 100°C for 30 minutes and followed by soaking the Sacha inchi beans in the boiled water for 24 hours. After soaking for 24 hours, steam for 15 minutes. After that, cool it by airing it until it reaches room temperature. After it cools to room temperature, yeast is added as much as 0.5% of the weight of the sacha inch seeds and mixed until evenly distributed. The packaging process uses banana leaves and is then fermented by aging and stored in incubator at a temperature of 20-37 °C for 72 hours[20].

In-*Vivo* Study

In vivo test was carried out at the Center for Food and Nutrition Studies Laboratory, Gadjah Mada University. A total of 36 Wistar rats were acclimatized for 7 days, then divided into 6 treatment groups with K0 as Healthy control and was not given treatment, K- as sick control without treatment, K+ as Drug control (Simvastatin 0.18 g), P1, P2, and P3 into treatment groups and given 0.9 Sacha Inchi Tempeh g, 1.8 g and 3.6 g respectively according to group. Then K-, K+, P1, P2, and P3 were given HFFD for 14 days. Next, sacha inchi tempeh intervention was given for 35 days. HFFD and intervention using the sonde method and standard rats feed using adlibitum method. This study has been declared feasible and passed the ethical test by the Ethical Comision Faculty of Medicine, Diponegoro University with number 004/EC-H/KEPK/FK-UNDIP/I/2024

Data Collection

Rats blood pressure was measured with a sphygmomanometer by placing the rat in a tube and measuring the blood pressure. The rat's body weight was measured by weighing the rat by placing the rat in a box and placing it on a calibrated scale. The body length of the rats was measured from the snout to the base of the tail. The Lee index calculation is calculated from body length and body weight using the formula:

$$\text{Lee Index} = \frac{\sqrt{\text{Body weight (g)x } 10}}{\text{Body length (mm)}}$$

Data Analysis

The research data will be tested for normality with Shapiro-Wilk and is said to be normal when the P value is >0.05. The next analysis is to carry out a homogeneity test and it is said to be homogeneous if the P value is >0.05. To see the effects before and after treatment, the Paired T-test was used and it was said to be significant if the P value <0.05. The next

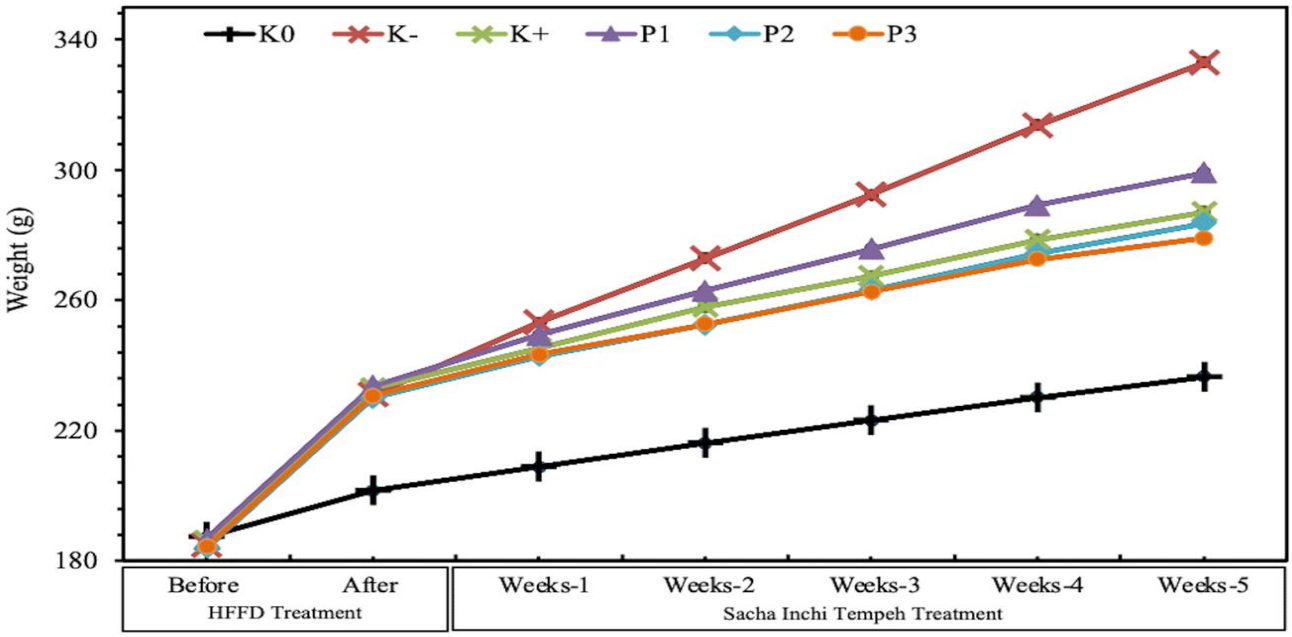
analysis is a one way-ANOVA test and it is said to be significant if the P value <0.05, then it will be continued with the LSD Post-Hoc Test to see the comparison of each group. Data analysis uses computer statistical applications.

RESULTS AND DISCUSSION

After acclimatizing the rats for 7 days, they continued with HFFD administration for 14 days and could be categorized as a metabolic syndrome because they met 4 of the 5 AHA (American Heart Association) criteria, namely Lee Index > 300, HDL < 40 mg/ dL, Triglycerides > 150 mg/dL, Blood Pressure >130 mmHg, and Fasting Blood Glucose > 100 mg/dL, so it can be concluded that the rats have experienced metabolic syndrome [21].

Figure 1, shows that rats before being given HFFD had a normal body weight, namely around 185.5 ± 3.54, but after HFFD their body weight increased drastically, reaching 231.6 ± 3.71. After giving HFFD, it was continued with sacha inchi tempeh intervention for 5 weeks and it was found that the greatest weight gain was in the K- group with body weight reaching 44%, P1 have a weight increase of 28%, P2 have a increase of 23.3%, P3 have an increase amounted to 20.9%, K+ have an increase of 23.3%, while K0 which was only given standard feed experienced an increase in body weight of 17.3%. This weight gain is in line with the length of maintenance and continues to increase.

Figure.1: Body weight Rats

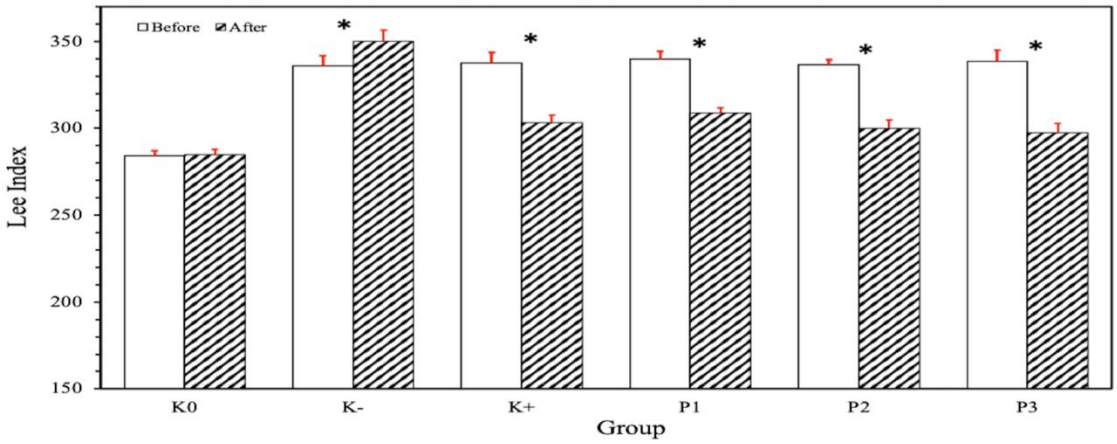


Fructose is a substitute for glucose or sucrose it is known that consuming high amounts of refined carbohydrates in food and beverages can increase the risk of dyslipidemia, obesity, and the occurrence of insulin resistance. High-fat diets can increase body weight, total fat, visceral fat, subcutaneous fat, insulin secretion, and fasting blood glucose levels [22]. A high-fat, high-fructose diet in the long term can increase the risk of developing obesity associated with metabolic disorders such as hyperglycemia, hyperinsulinemia, insulin resistance, glucose intolerance, and dyslipidemia (hypertriglyceridemia and hypercholesterolemia) [23].

In the Lee Index, it is shown in Figure 2 that changes occurred after the sacha inchi tempeh intervention. The small decrease in the lee index was in group P1 with a decrease of 9.1%, and the largest decrease was in group P3 with a decrease of 12%, while K+ only had a decrease of 10.1%. In the K- group there was no decline and tended to continue to increase, while in the K0 group, there was no significant change. After being tested with a paired T-test, it shows that there is a significant difference in K- (p 0.000) which can be seen in Figure 2, there is an increase in the Lee index, and significant differences are also experienced in the K+ (p 0.000), P1 (p 0.000), P2 (p 0.000) and P3 (p 0.000), while K0 does not have a significant difference.

Tempeh has antioxidants in the form of isoflavones, akin flavonoid which act as inhibitors of the formation of free radicals. Antioxidants in tempeh are formed during the fermentation process. The flavonoid in tempeh are hypocholesterolemic so that the polysaturated fatty acid content in tempeh can reduce cholesterol levels [24]. Isoflavones can reduce weight or increase insulin levels. In addition, flavonoid have an important role in modulating serum glucose levels in diabetic rats. Complex compounds in fermented tempeh that are metabolized by microorganisms can produce compounds with higher nutritional value, such as an increase in aglycones during the fermentation process [25]. Tempeh fermentation can increase antioxidant activity and can break down peptides in sacha inchi so that it can provide benefits such as increasing digestibility and having a good impact on improving the glycemic index in rat metabolic syndrome [26–28].

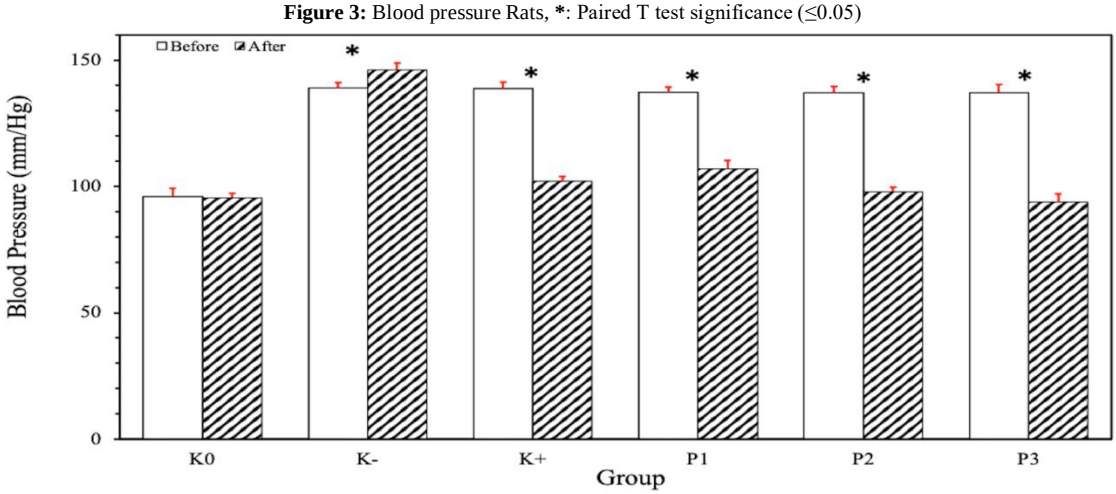
Figure.2: Lee index Rats, *: Paired T test sigmifican (≤0.05)



Increasing the amount of dietary fiber intake such as consuming nuts, vegetables, fruit and whole wheat products can help reduce triglyceride levels, increase HDL-C levels, improve blood pressure, control body weight and glycemia. Sacha inchi is a type of legume that contains insoluble dietary fiber which plays a role in helping protein absorption, reducing weight, and reducing insulin resistance and can consistently reduce the risk of diabetes mellitus and obesity [29].

In Figure 3, changes in blood pressure are shown after the sacha inchi tempeh intervention for 5 weeks. It can be seen that there was a decrease in blood pressure in all intervention groups, with the smallest decrease in blood pressure being in P1 with a decrease of 22%, and the largest in group P3 with a decrease in blood pressure of 29.7%. While K+ had a decrease of 26.5%. For the K- group, namely the group without intervention, there was an increase in blood pressure and K0 did not experience a significant change. After being tested with the Paired T-test, it showed that there was a significant difference before and after in all sacha inchi tempeh intervention groups, namely at P1, P2, and P3 there was a significant difference (p 0.000). The K- group had a p-value of 0.007 because they experienced a drastic increase in blood pressure, K+ had a p-value of 0.000 and K0 had no significant difference because it had a p-value of 0.784, which means it was not significant (>0.05).

Figure 3: Blood pressure Rats, *****: Paired T test significance (≤0.05)



In Table 1, there is a significant difference after the ANOVA test and has a p-value of 0.000 in body weight, Lee index, and Blood Pressure. P3 had the greatest decline when compared with other groups because the difference was the largest. When compared between groups, there was an insignificant difference between K+ and P2 so giving 1.8 g of Sacha inch tempeh was the same as giving medication.

Table.1: Result of Anova weight, Lee index and Blood Pressure

Group	Δ Before and After Intervention		
	Weight	Lee Index	Blood Pressure
K0	35.0 ± 1.26^{a}	0.65 ± 0.41^{a}	0.5 ± 4.23^{a}
K-	101.8 ± 5.91^{b}	14.1 ± 2.06^{b}	7.1 ± 3.97^{b}
K+	54.3 ± 1.21^{c}	$34.2 \pm 1.90^{c,d}$	36.8 ± 3.37^{c}
P1	65.5 ± 1.83^{d}	31.0 ± 1.33^{c}	30.3 ± 3.98^{d}
P2	53.6 ± 0.83^{c}	36.4 ± 6.42^{d}	$39.0 \pm 3.94^{c,e}$
P3	48.3 ± 1.86^{e}	40.8 ± 1.38^{e}	43.3 ± 4.92^{e}
*p**	0.000	0.000	0.000

*: one-way ANOVA; (a,b,c,d,e): Post-Hoc LSD

These results are in line with research conducted by Yi En et al (2024) which revealed that giving sacha inchi oil 1.6 g for 2 months caused improvements in blood pressure [7]. In another study conducted by Gonzales et.al (2014) which showed that giving 15 ml of sacha inchi oil for 4 months had a blood pressure lowering effect [9]. This effect is because PUFA such as ALA & LA can have an effect on lowering LDL cholesterol which is related to the development of hypertension. Various studies regarding the relationship between LDL and blood pressure have been widely discussed [31–33]. Enzyme (ACE), which was confirmed in research by Chirinos et al (2020) after administration of sacha inchi protein hydrolyzate at 98 μg/mL.[34] This is because sacha inchi has a phenolic effect and high antioxidant activity. Hypertension occurs because there is abnormal calcium regulation in the kidneys, this occurs due to increased regulation of calcium pathways and increased expression of L-type calcium channels (LTCC) which causes the entry of calcium into the blood vessels which will cause vasoconstriction which causes hypertension [37].

Sacha Inchi Tempeh also increases Na+/K+ATPase which will improve sodium and potassium homeostasis [38]. Miss regulation of sodium and potassium is a major factor in the pathogenesis of hypertension. The Na+/K+-ATPase pump is important for maintaining sodium electrochemistry across cell membranes. In hypertension, Na+/K+-ATPase expression is decreased, which contributes to sodium retention and plasma volume expansion [39-40]. In addition, the mechanism for lowering blood pressure in sacha inchi tempeh is related to the regulation of NO and eNOS which are related to hypertension. One significant vasoactive molecule that affects blood pressure and vascular function is NO, which is produced by eNOS. NO bioavailability is decreased by superoxide's constant emission above the body's natural antioxidant capacity [41,42]. Decreased NO levels cause endothelial dysfunction, impaired vasodilation, and increased blood pressure [43]. This mechanism is related to Sacha Inchi Tempeh which can increase levels of 5-methyltetrahydrofolate (5-MTHF), an active form of di-tetrahydrofolic acid [24]. 5-MTHF inhibits the synthesis of superoxide to preserve NO bioavailability [44].

The reduction in obesity status as evidenced by the Lee Index <300 is also supported in various previous studies, ALA and LA in the body are regulated and converted into EPA and DHA [45]. The effect of PUFA in improving obesity was proven in Mayer et.al (2019) research which provided Marine Microalga which is high in PUFA, EPA, and DHA which can reduce body weight for 8 weeks [46]. This may be related to the effects of ALA and LA which can reduce triglyceride synthesis [47]. ALA and LA are unsaturated fatty acids contained in sacha inchi which will interact with the peroxisome proliferator active receptor alpha (PPARα) which in turn interacts with the peroxisome proliferator response element (PPRE) and lipoprotein lipase (LPL) which is an enzyme that can hydrolyze triglycerides. ALA and LA will stimulate Lipolysis, which hydrolyzes triglycerides from chylomicrons and cholesterol. Apart from that, ALA also reduces ApoC-III which is an inhibitory factor for Lipoprotein Lipase. By inhibiting fatty acid synthase, acetyl coenzyme A (CoA) carboxylase, and diacylglycerol acyltransferase, ALA and LA can lower triglycerides [48].

Apart from reducing triglyceride synthesis, perhaps this mechanism is related to a decrease in the hormone leptin and adiponectin. Sacha Inchi can reduce the hormones leptin and adiponectin and improve obesity status in Ambulay et al (2019) study after administering sacha inchi oil emulsion for 11 weeks. The leptin hormone is a hormone that can reduce appetite and regulate body weight [49]. Rats become obese after being induced by HFFD. In the event of obesity, leptin resistance occurs, causing increased food consumption and inflammation [50]. Meanwhile, adiponectin is an adipokine that has anti-inflammatory properties, but its levels decrease in line with the incidence of obesity [51]. The positive effect of sacha inchi tempeh on the hormones leptin and adiponectin is mediated by increasing the expression of peroxisome proliferator-activated receptor alpha (PPAR-α), a transcription factor that affects oxidative stress and fatty acid metabolism [52].

Other research states that the administration of sacha inchi oil causes an increase in the secretion of the liver lipase enzyme.[53] An essential lipolytic enzyme that supports lipoprotein absorption in the de novo lipid production pathway is hepatic lipase [54]. Moreover, SI oil inhibits the synthesis of proinflammatory phospholipids called lysophosphatidylethanolamine (LysoPE) and lysophosphatidylcholine (LysoPC), which are found in people with obesity and hyperlipidemia brought on by HFD [55]. Additionally, in HFD-fed animals, SI oil reduced the expression of phosphatidylglycerol phosphate synthase 1 (PGS1) in the liver [53]. A strong lipolysis inhibitor, phosphatidylglycerol, is produced via the catalysis of PGS1. Consequently, SI oil's suppression of PGS1 lowers the synthesis of phosphatidylglycerol and enhances lipolysis [53].

CONCLUSION

It can be concluded from this discussion that sacha inchi tempeh has an improving effect on the blood pressure and Lee index of rats. The most effective group was P2 (1.8 Sacha inch tempeh) because it had no difference to the drug simvastatin and was able to reduce blood pressure and Lee index to the normal category.

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Conflict of interest

The authors declare that they have no interest whatsoever in the preparation of this manuscript.

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