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**Research Article**

# The Effect of Sacha Inchi Tempe on Blood Glucose, HOMA-IR, and TNF-α in Rats with Metabolic Syndrome

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## ABSTRACT

This research aimed to evaluate the impact of sacha inchi tempe (*Plukenetia volubilis* L.) on Fasting Blood Glucose (FBG), Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), and Tumor Necrosis Factor Alpha (TNF-α) levels. In addition, metabolic syndrome was induced in 36 male Wistar rats aged 2 months at 150–200 g weight by giving a High-Fat High-Fructose Diet (HFFD) for 2 weeks. The extract was administered through oral gavage in dose-dependent manner and rats were allocated into 6 groups, namely: 1). Normal control or K0; 2). Negative control or K-; 3). Positive control or K+ with 0.18 mg/200 g BB of simvastatin; 4). Intervention with 0.9 g sacha inchi tempe or P1; 5). Intervention with 1.8 g sacha inchi tempe or P2, and; 6). Intervention with 3.6 g sacha inchi tempe or P3. Meanwhile, normal chow rats were used and served as the control group. After 2 and 5 weeks of induction and intervention, blood was drawn to determine FBG. Blood insulin was examined after 5 week of intervention. Rats were euthanized at the end of the intervention for hepatic TNF-α analysis before calculating HOMA-IR. The result showed that there was a significant difference ($p<0.05$) in FBG, HOMA-IR and hepatic TNF-α levels after sacha inchi tempe treatment. Rats receiving the highest dose of sacha inchi tempe had the most significant reduction ($p<0.05$) in FBG, HOMA-IR and hepatic TNF-α, when compared to simvastatin group. Therefore, sacha inchi tempe could attenuate glycemic and inflammation profiles in metabolic syndrome.

## INTRODUCTION

Metabolic syndrome is a group of abnormalities, including insulin resistance, hypercholesterolemia, hypertriglyceridemia, hypertension, central obesity, and low High-Density Lipoprotein Cholesterol (HDL-c). In this context, the risk of cardiovascular disease and type 2 diabetes mellitus can be increased twofold and fivefold, respectively (Wang *et al.* 2020). The prevalence of metabolic syndrome is increasing worldwide and has reached 21.66% in Indonesia (Herningtyas & Ng 2019).

The pathophysiology of metabolic syndrome starts with genetic factors, increased calorie intake containing simple sugar and high fat, and low physical activity. This causes the accumulation of visceral fat which increases Reactive Oxygen Species (ROS) and Renal Artery Stenosis (RAS), hence triggering an increase in angiotensin II. Similarly, inflammatory factors such as IL-6, TNF-α, CRP, and fibrinogen, are increased. Free Fatty Acids (FFA), lipogenesis, gluconeogenesis, and triglycerides are increased while insulin and glucose uptake are decreased. Hyperglycemia conditions also increase lipid

production which is accumulated in the liver to enhance insulin resistance (Rojanaverawong *et al.* 2023; Wang *et al.* 2016).

Sacha Inchi is a tropical plant rich in oil (35%–60%), protein (25%–30%), minerals, essential amino acids, and vitamin E (Torres-Sánchez *et al.* 2023). The major fatty acid compounds in this plant are linolenic and linoleic acids. The α-Linolenic Acid (ALA) is an essential fatty acid found in the ω-3 group. ALA serves as a substrate for the production of Docosapentaenoic Acid (DHA), Eicosapentaenoic Acid (EPA), and other longer-chain of unsaturated ω-3 fatty acids (Baker *et al.* 2016). The compound has antioxidant activity by increasing the GSH/GSSG ratio and GSH content without modification of the levels in the liver (Rincón-Cervera *et al.* 2016). Meanwhile, sacha inchi has the highest ALA compared to other tropical nuts such as peanuts, cashews, tropical almonds, and Brazil nuts. The content is 10–100 times higher than other nuts, hence foods made from the plant contain high levels of ALA (Cardoso *et al.* 2017; Ng *et al.* 2015; Rico *et al.* 2016; USDA 2021).

Tempe is a traditional Indonesian food prepared by fermenting soybeans using *Rhizopus spp.* The fermentation process causes tempe to have several advantages such as a reduction in anti-nutritional substances, elevation in vitamins and high bioavailability of carbohydrates, proteins, and fats (Ahnan-Winarno *et al.* 2021; Bueno-Borges *et al.* 2018; Nurrahman *et al.* 2013). The food contains genistein, daidzein, and β-sitosterol, which influence blood glucose balance and prevent cancer, heart disease, and type 2 diabetes (Huang *et al.* 2018). Previously, Ulfa *et al.* (2022) made tempe from kedawung seeds which improved hemoglobin and albumin in protein-energy deficient rats. In addition, sorghum tempe was reported to reduce Low-Density Lipoprotein-cholesterol (LDL-c) and malondialdehyde levels in rats (Khoirun Nisa *et al.* 2021). Sacha inchi tempe contains 1.97% ash, 28.51% moisture, 10.06% carbohydrates, 38.43% fat, 20.50% protein, 1.60% Saturated Fatty Acids (SFA), 2.68% Monounsaturated Fatty Acids (MUFA), 32.99% Polyunsaturated Fatty Acids (PUFA), 14.18% Linoleic Acid (LA), and 18.82% α-Linolenic Acid (ALA). The best duration of the fermentation is 72 hours but there is limited research on sacha inchi tempe (Salam *et al.* 2023). Moreover, sacha inchi is used as an additional ingredient in making yogurt. Partial substitution of cow's milk with the seeds significantly increases ALA in yogurt (Vanegas-Azuero & Gutiérrez 2018). Tempe and other fermented soybean products, such as natto, miso, kinema, doenjang, douchi, and chungkookjang, can improve metabolic syndrome (do Prado *et al.* 2022).

Sacha inchi tempe has the potential against metabolic syndrome due to the bioactive ingredients. Therefore, this research aims to determine the effect of giving sacha inchi tempe on Fasting Blood Glucose (FBG), insulin resistance (HOMA-IR), and pro-inflammatory factor TNF-α in male Wistar rats with metabolic syndrome.

## METHODS

### Design, location, and time

This experimental research was carried out with a randomized post-test control group design. Sacha Inchi seeds were obtained from Kuningan, West Java, Indonesia and the research on experimental animals was carried out in a facility at the Center for Food and Nutrition Studies, Gadjah Mada University, D.I. Yogyakarta from January to March 2024. The ethical clearance was obtained by The Research Ethics Committee of Faculty Medicine, Diponegoro University No. 003/ EC-H/ KEPK/FK-UNDIP/I/2024.

### Materials and tools

Sacha inchi tempe as treatment was made by researchers from sacha inchi seeds and "Raprima" brand mold. Induction of metabolic syndrome was done by administering a High-Fat and Fructose Diet (HFFD) for 2 weeks. The diet given was 15 g–20 g/day (10% of body weight), consisting of pork fat (20%), cholesterol (1.5%), cholic acid (0.5%) and fructose as much as 1 mL/200 g per body weight of rats (65% fat, 25% carbohydrate, and 10% protein). Lee's index was measured using a Medline and a digital scale. Triglycerides were measured by Triglycerides GPO FS Kit (DiaSys, Germany). HDL-c was measured by Cholesterol CHOD FS Kit (Diasys, Germany). The German-made S-2 sphygmomanometer was used to measure blood pressure using the tail-cuff method. FBG was measured by Glucose GOD FS Kit (DiaSys, Germany). Insulin levels were measured by insulin ELISA Kit (FineTest, China). TNF-α levels were measured by TNF-α ELISA Kit (FineTest, China).

The tools used in this research were cages, food dish, drinker, Eppendorf, 0.5 ml EDTA tube, oral gavage, syringe, microhematocrit, water bath, centrifuge, cooling bath, microplate reader, Uv-Vis spectrophotometer, and cuvette.

**Procedure**

***Sacha inchi tempe.*** Sacha inchi seeds were washed thoroughly and soaked for 2 hours. After soaking, sacha inchi seeds were boiled for 30 minutes and then resoaked for 24 hours until mucus appeared on the surface of the water. Subsequently, the seeds were drained and cleaned with the attached epidermis. Sacha inchi seeds were steamed for 15 minutes, spread evenly in a container, cooled, dried, and sprinkled with 0.5% tempe yeast of the total weight. The seeds were packaged using banana leaves and stored in a dark, damp place for 72 hours (Salam *et al.* 2023).

***Animal research.*** According to the result obtained, thirty-six male Wistar rats weighing178.31±3.40 g were used. In this context, the preadult and adult ages were represented by 2-month-old rats (Fitria *et al.* 2018) and the acclimatization process was carried out for seven days in 12 light-dark cycles with a temperature control of 25±2°C. Additionally, diet of 15 g−20 g CP594 feed were administered with water and randomly assigned to six groups. This research comprised normal control (K0), metabolic syndrome (K-), simvastatin (K+), sacha inchi tempe dose I (P1), sacha inchi tempe dose II (P2), and sacha inchi tempe dose III (P3). Groups K-, K+, P1, P2, and P3 received 15 g/day of HFFD for 14 days in the process of inducing metabolic syndrome, while K0 was given standard diets and ad libitum water. The three components of metabolic syndrome included obesity (Lee Index >300), triglyceride levels >150 mg/dL, HDL levels <40 mg/dL, FBG levels >100 g/dL, and systolic blood pressure >130 mmHg (Srikanthan *et al.* 2016). After 14 days, group K- was given standard feed only, group K+ was given standard feed and simvastatin (0.18 mg/200 g BW/day), group P1, P2, and P3 received standard feed + 0.9, 1.8, and 3.6 g/200 g BW/day sacha inchi tempe, respectively. The extract was grinded before adding distilled water to obtain a 5 mL volume once a day for 5 weeks through oral gavage and the rats fasted for 8 hours before the blood was drawn to determine FBG and insulin levels. Meanwhile, the tissue for hepatic TNF-α levels was obtained by euthanizing the sample with the ketamine overdose method.

Blood was extracted twice through the orbital sinus using the retro-orbital plexus method after 14 days of HFFD and 5 weeks of tempe intervention. Meanwhile, the serum was obtained by leaving blood at room temperature and centrifuging for 15 minutes at 4,000 rpm. Liver tissue was taken at the end of the research to check TNF-α levels.

***Metabolic profile analysis.*** GOD-PAP, Enzymatic Calorimetric Test of Glucose Oxidase Phenol 4-Aminophenazone were used to obtain FBG levels (Subiyono *et al.* 2016). Additionally, insulin levels were reported by reacting serum with monoclonal anti-rat insulin (antibodies) coated in microplate wells and the reagents in the ELISA Kit. The analytical process was carried out according to the kit's instructions (Shen *et al.* 2019) while Lee index calculation was obtained from the weight and length of rats. The weight was determined by weighing rats using a digital scale. Similarly, the length of the bodies was measured from the snout to the base of the tail using a Medline. Lee index was calculated using the following formula:

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

Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) measurement. HOMA-IR is used in blood test results to assess insulin resistance formulated as (fasting insulin × fasting glucose) / 405, with glucose and insulin in units of mg/dL and µIU/mL, respectively (Putri *et al.* 2022).

TNF-α levels measurement. TNF-α levels were measured using liver tissue and a total of 0.1 g was mixed with 1 mL of PBS pH 7.2 then crushed using a mortar and centrifuged at 5,000 rpm for 5 minutes. The supernatant was transferred to a microtube and the measurement was carried out using the sandwich ELISA technique in line with the protocol specified for the kit (Sholihah *et al.* 2018).

**Data analysis**

The mean and standard deviation of all the collected data were reported using SPSS (IBM, version 22). Statistical tests were carried out to determine the differences before and after the intervention in FBG. In addition, the paired t-test was adopted for the FBG before and

after intervention. One-way ANOVA statistical test, followed by posthoc Tukey HSD with a significant value of $p<0.05$ was carried out to determine the difference of FBG, HOMA-IR, and TNF-α among groups.

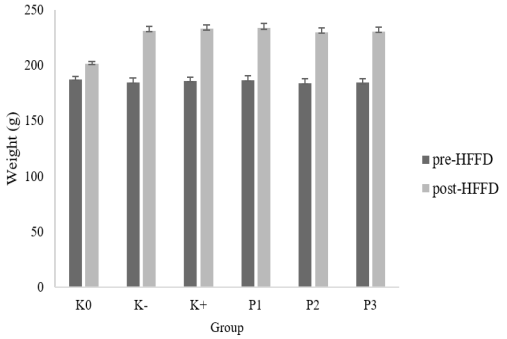
## RESULTS AND DISCUSSION

A total of 36 rats were divided into six groups since no death was recorded during the intervention. The results showed that sacha inchi tempe significantly lowered FBG levels, insulin resistance as measured by HOMA-IR index, and TNF levels seen in Figure 2. Sacha inchi tempe was able to increase insulin levels in metabolic syndrome rats.

According to Salam *et al.* (2023), the optimum fermentation time was 72 hours and sacha inchi Tempe contained 18.82% ALA. In this research, 0.9 g, 1.8 g, and 3.6 g of sacha inchi tempe were equivalent to 169.38 mg ALA, 338.76 mg ALA, and 677.52 mg ALA, respectively.

***Body weight.*** Group K0 was not given HFFD induction while the other 5 groups received the induction. There were changes in the body weight and the greatest increase in the HFFD-induced group was the K+ group. K0 showed the smallest increase in body weight because the group was not induced by HFFD. The weight gain in K0 was lower when compared to the control in Battung *et al.* (2019) with a weight gain of 22.6 g due to the age difference. In addition, the standard deviation value was relatively large compared to the results. Groups K-, K+, P1, P2, and P3 showed a higher increase in body weight compared to K0 ranging from 46–47 g in each group given HFFD. The results showed a greater increase in body weight compared to research using a hypercholesterol diet and 60% fructose, which gave an increase of about 30 g in 2 weeks (Barrios-Ramos 2014). Hussain *et al.* (2019) stated that weight gain was increased by 3.4g/day and 8g/day in the control and HFFD, respectively. On the 14th day, the weight change of the control and HFFD groups were 47.6 g and 112 g, respectively. A statistical test with one-way ANOVA showed no significant difference in the pre-HFFD group, hence the sample had been randomized successfully. However, these results showed the success of HFFD induction on body weight, as reported in Figure 1.

***Metabolic syndrome biomarkers.*** After HFFD administration for 2 weeks, rats in the K-,




K0: Healthy + standard diet; K-: Metabolic syndrome + standard diet; K+: Metabolic syndrome + 0.18 mg/200 g b.w./day of simvastatin; P1: Metabolic syndrome + 0.9 g sacha inchi tempe; P2: Metabolic syndrome + 1.8 g sacha inchi tempe; P3: Metabolic syndrome + 3.6 g sacha inchi tempe

**Figure 1. The mean weight of rats before and after High-Fat High-Fructose Diet (HFFD) induction**

K+, P1, P2, and P3 groups experienced an increase in Lee Index, HDL-c, FBG, and systolic blood pressure, as well as a decrease in HDL-c. This research showed a higher Lee index in the HFFD group compared to El-Saka who used 3–4 weeks-old rats (El-Saka *et al.* 2023). Based on Zhang in Sprague Dawley rats fed HFFD for 20 weeks, the HDL-c value was 48 mg/dL (Zhang *et al.* 2022). However, the results are in line with Hidayati who experienced a significant decrease in HDL-c after administration of HFFD (Hidayati *et al.* 2020). Hypertriacylglycerolemic rats fed HFFD for 5 weeks showed a systolic blood pressure of 133 mmHg, lower than the results. However, rats experienced hypertension (Sasváriová *et al.* 2019) and higher FBG was reported after HFFD administration. Previous research showed a normal HFFD of 84 mg/dL after 2 weeks. Hidayati who gave HFFD for 4 weeks reported a significant increase in FBG (Hidayati *et al.* 2020) but the level of triglycerides did not improve after the administration. Based on Barrios-Ramos triglycerides increased when HFFD was given for 7 weeks. Even though HFFD was given for 2 weeks, TG was 57 mg/dL, lower than any group (Barrios-Ramos 2014). However, rats had fulfilled >3 of the 5 metabolic syndrome criteria and the condition was achieved (Srikanthan *et al.* 2016). The differences were due to the type and age of rats, as well as the duration of HFFD, as presentend in Table 1.

**Table 1. The mean biomarker of Lee index, TG, HDL-c, FBG, and blood pressure after HFFD induction**

| Biomarker | Normal value | Groups of treatment (Mean±SD) | | | | | |
|---|---|---|---|---|---|---|---|
| | | K0 | K- | K+ | P1 | P2 | P3 |
| Lee index | <300 | 284.11±3.01 | 335.99±5.81 | 337.46±6.08 | 339.85±4.28 | 336.55±2.98 | 338.31±6.66 |
| TG (mg/dL) | <150 | 70.91±2.48 | 136.04±2.56 | 135.57±2.34 | 134.16±1.97 | 133.69±3.11 | 132.16±3.31 |
| HDL-c (mg/dL) | >40 | 82.65±1.27 | 23.58±2.38 | 23.81±2.15 | 24.72±1.47 | 23.47±1.27 | 23.24±1.69 |
| FBG (mg/dL) | 70–100 | 71.73±2.87 | 148.65±2.41 | 149.26±2.22 | 151.22±1.75 | 150.34±2.63 | 148.79±1.37 |
| Systolic blood pressure (mmHg) | <130 | 96.00±3.41 | 139.00±2.10 | 138.83±2.48 | 137.33±2.07 | 137.00±2.68 | 137.17±3.19 |

K0: Healthy + standard diet; K-: Metabolic syndrome + standard diet; K+: Metabolic syndrome + 0.18 mg/200 g b.w./day of simvastatin; P1: Metabolic syndrome + 0.9 g sacha inchi tempe; P2: Metabolic syndrome + 1.8 g sacha inchi tempe; P3: Metabolic syndrome + 3.6 g sacha inchi tempe; TG: Triglyceride; HDL: High-Density Lipoprotein; FBG: Fasting Blood Glucose; HFFD: High-Fat High-Fructose Diet; SD: Standard Deviation

***Caloric restriction.*** The K-, K+, P1, P2, and P3 groups were given a normal diet during the 5 weeks of the intervention period. Apart from the intervention received in the form of simvastatin or tempe, these rats were given caloric restriction because HFFD was stopped.

***FBG levels.*** The administration of HFFD for 14 days increases FBG. Exposure to fructose indirectly creates compensatory hyperinsulinemia. This condition includes fructose transporter (GLUT5), which contributes to insulin resistance and raised plasma glucose concentrations (Barrios-Ramos 2014). Since fructose has lipogenic (fat-producing) properties, a considerable amount enters the liver and accumulates as triglycerides and cholesterol. This reduces insulin sensitivity and increases the resistance and glucose intolerance. A high-fat diet contributes to increasing triglycerides and causes insulin resistance (Wong *et al.* 2016). In this research, triglycerides were not higher than 150 mg/dL, hence fructose played a greater role in increasing FBG.

There was a significant difference in each group before and after 5 weeks of intervention ($p<0.001$), as reported in Figure 2A. In addition, the level of FBG was increased in the K+, P1, P2, and P3 groups. This shows that the K+ group as well as P1, P2, and P3 reduce FBG values. From the post hoc test, the difference in FBG value (ΔFBG) between K0 and K- is not significant. This is because the two groups were not given simvastatin or sacha inchi tempe. In the K- group and those given the simvastatin/sacha inchi tempe intervention, the FBG value increased and decreased, respectively. This showed the effect of the intervention on reducing FBG levels. Groups P1 and P2 were not significant, while P3 was significant from P1 and P2. In addition, groups P1, P2, and P3 were not significantly different compared to the simvastatin group (K+).

FBG levels in metabolic syndrome rats group increased compared to K0. Metabolic syndrome could trigger insulin resistance, inflammation, and oxidative stress to increase FBG levels (Fahed *et al.* 2022). Meanwhile, a tight range of glucose is maintained to ensure a steady supply. This is because the liver is the primary organ responsible for controlling blood glucose, insulin, glucagon, and epinephrine (König *et al.* 2012).

Simvastatin group (K+) reduced FBG significantly compared to those without intervention (K-). In addition, simvastatin is a member of statin, an HMG-CoA reductase inhibitor used in the treatment of metabolic syndrome (Chan & Watt 2011). This FBG-decreasing effect can be caused by simvastatin in reducing MDA and increasing GSH (Crespo & Quidgley 2015; Hadi *et al.* 2015). The antioxidant effect plays a role in decreasing HOMA-IR values. The results are in contrast to Fawzy Fahim where the administration of streptozotocin-induced diabetic rats did not significantly reduce FBG (Fawzy Fahim *et al.* 2019).

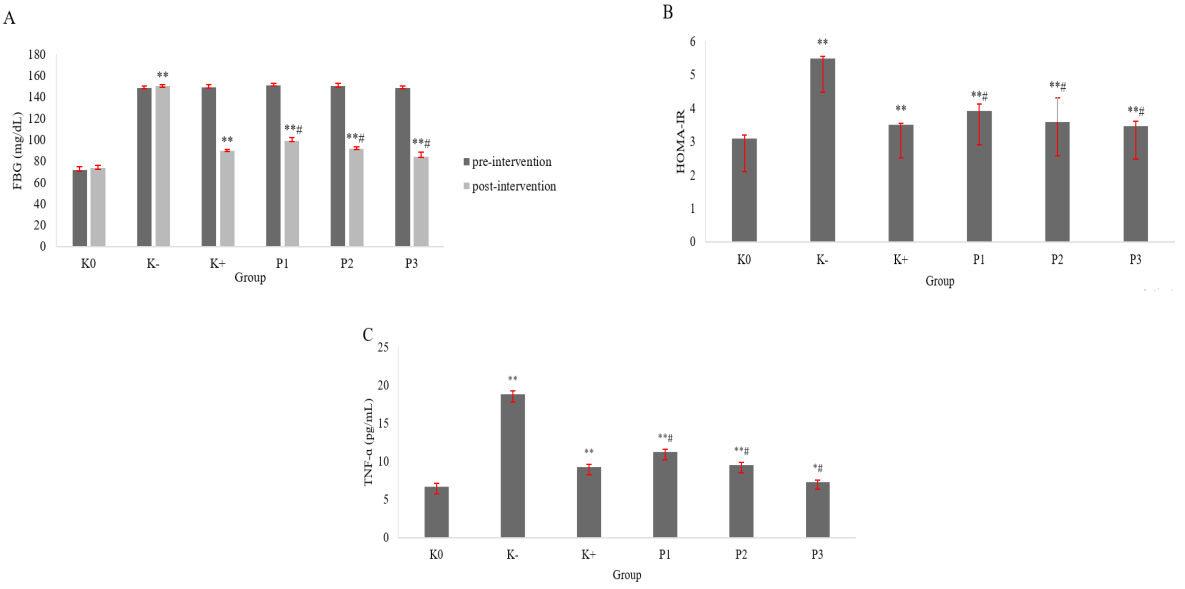
P1, P2, and P3 groups decreased FBG levels significantly when compared to K-. Sacha inchi tempe is rich in ALA and flavonoids used to suppress enzymes in gluconeogenesis such as Phosphoenol Pyruvate Carboxykinase (PEPCK) and G-6-Pase. This is the first research to discuss the effect of sacha inchi tempe on FBG.

Rojanaverawong reported that 1 mL/kg of the extract reduced FBG from 333 mg/dL to 225 mg/dL in 5 weeks (Rojanaverawong *et al.* 2023).

***HOMA-IR.*** HOMA-IR was used to assess insulin resistance, by calculating the concentration and fasting plasma glucose in the context of diabetes mellitus (Putri *et al.* 2022). The value after sacha inchi intervention is shown in Figure 2B and there was a significant difference in the mean between groups ($p<0.001$). Meanwhile, insulin resistance and HOMA-IR values were affected by discontinuation of HFFD administration in rats receiving intervention (Barrios-Ramos 2014). HOMA-IR values of the intervention group P1, P2, and P3 were significantly different from K-. In this context, insulin resistance was reduced by the improvements in HOMA-IR values and sacha inchi tempe. Simvastatin group and P2 were not significantly different since P3 was better in reducing HOMA-IR value. Therefore, 3.6 g sacha inchi tempe had a better effect than simvastatin on HOMA-IR value in metabolic syndrome conditions. In the intervention group, P3 had the lowest HOMA-IR value, followed by P2 and P1. There were significant differences between groups P1, P2, and P3, hence the effect was dose-dependent. As the best dose, P3 reduced HOMA-IR value by 38.3% compared to the negative control. The results were in line with Rojanaverawong who showed a decrease in rats given sacha inchi oil. In this context, 1 mL/kg of sacha inchi oil reduced HOMA-IR value to 9 (Rojanaverawong *et al.* 2023).

A high HOMA-IR index signifies a disturbance in the body cells' absorption. The cafeteria diet commonly consumed tends to be high in sugar and fat, increasing visceral fat and triggering inflammation through increased TNF-α, IL-6, and decreased IL-10. In addition, there was an increase in FFA which causes oxidative stress. Insulin resistance may result from disruptions to signalling caused by oxidative damage and inflammation (Ahmed *et al.* 2022).

The decrease in HOMA-IR values after sacha inchi tempe intervention showed a repair response of target cells to activate the use of glucose. Sacha inchi tempe contained ALA, flavonoids, and β-sitosterol which could improve insulin sensitivity through the IRS-1/PI3K/Akt signaling pathway. This is achieved by increasing IRS-1 and p-Akt (Ser 473) protein expression in the liver (Rojanaverawong *et al.* 2023). The insoluble dietary fiber triggered the formation of acetate which played a role in improving insulin




Data are expressed as mean±standard error of the mean (n=6)
*p<0.05; **p <0.001 vs. the control group; #p <0.001 vs. the diabetic group, as analyzed with ANOVA followed by Tukey's HSD test
P: Treatment; K+: Simvastatin; K-: Diabetic group; K0: Normal control

**Figure 2. The effect of sacha inchi tempe on (A) fasting blood glucose, (B) homeostatic model assessment for insulin resistance, and (C) tumor necrosis factor-alpha**

sensitivity (Fu *et al.* 2022; González Hernández *et al.* 2019). Sacha inchi reduced triglycerides and triggered the formation of Very Low Density Lipoprotein (VLDL). In diabetic rats, sacha inchi oil decreased blood and pancreatic MDA levels as well as atrophic pancreatic islets (Wongmanee *et al.* 2024).

***Hepatic TNF-a levels.*** Hepatic TNF-α levels in Figure 2C showed a significant difference in values between the treatment groups (P1, P2, and P3) and DM ($p<0.001$). Tempe treatment groups P1, P2, and P3 were significantly different from the K- group. This showed that administration of sacha inchi tempe reduced hepatic TNF-α levels. Meanwhile, P2 group was not significantly different from K+ which received simvastatin intervention in reducing hepatic TNF-α. P3 was better at reducing TNF- α levels compared to K+. In this context, 3.6 g sacha inchi tempe had a better effect than simvastatin on TNF-α levels in metabolic syndrome conditions. Conversely, P3 had the smallest levels of TNF-α, followed by P2 and P1. The group had an amazing effect on reducing hepatic TNF-α which caused a reduction of 61% compared to the negative control, while K+, P2, and P1 were reduced by 50.7%, 49.2%, and 40.1%, respectively. Groups P1, P2, and P3 had significant differences since sacha inchi tempe reduced TNF-α in a dose-dependent manner.

TNF-α is a cytokine produced in adipose tissue by macrophages. The production is proportional to adipose tissue mass and is related to insulin resistance. In addition, insulin resistance is influenced by the following mechanisms: 1). Inhibiting GLUT4 expression; 2). Stimulating lipolysis and increasing FFA levels; 3). Inhibiting insulin signaling through serine phosphorylation of IRS-1; 4). Inhibiting the synthesis of Peroxisome Proliferator-Activator Receptor γ (PPARγ) (Moller 2000).

The group receiving simvastatin experienced a decrease in TNF-α levels in line with another research that gave simvastatin 20 mg/day to peritoneal dialysis patients. The decrease in TNF-α was caused by the reduction in LDL-c levels. Meanwhile, the decrease in LDL-c caused a reduction in TNF-α production by endothelial cells, macrophages, and smooth muscle cells (Tugrul Sezer *et al.* 2007). Sacha inchi oil (1 mL/kg) reduces TNF-α by 2.9 pg/mL, which is lower than the result (Rojanaverawong *et al.* 2023).

Sacha inchi as the main component in tempe contains active ingredients such as ALA, α-tocopherol, and flavonoids showing anti-inflammatory properties in diabetic rats (Ghadge *et al.* 2016; Jamalan *et al.* 2015; Othman *et al.* 2021). In a research carried out by Rojanaverawong *et al.* (2023), the administration of sacha inchi significantly improved inflammatory cytokines and liver inflammation while maintaining the function and increasing insulin sensitivity (Ambulay *et al.* 2020). Moreover, oxidative stress and TNF-α expression resulted from ROS-mediated activation of p38 mitogen-activated protein kinase and NF-κB, triggering the release of extra TNF-α (Grandl & Wolfrum 2018). The limitation of this study is sacha inchi tempe was not compared with the seeds so that the effect of fermentation on the extract could not be determined.

## CONCLUSION

In conclusion, a significant effect was shown by sacha inchi tempe in reducing FBG, HOMA-IR, and TNF-α levels. The best dosage for improving metabolic health of rats was a dose of 3.6 g. Since liver and pancreas were closely related to the glycemic profile, further research should be conducted to examine the histology of the organs.

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## DECLARATION OF CONFLICT OF INTERESTS

The authors have no conflict of interest.

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