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RESEARCH ARTICLE

Restoration of Brain Insulin Signaling by *Tinospora crispa* Extract via PI3K/AKT Pathway Modulation in HFHF-Fed Rats

Kartika Rahma[1,2], Erni Hernawati Purwaningsih[3,*], Yetty Ramli[4] and Aulanniam Aulanniam[5]

[1]*Doctoral Program in Biomedical Sciences, Faculty of Medicine, Universitas Indonesia, Jakarta 10430, Indonesia*
[2]*Department of Pharmacy, Faculty of Health Science and Technology, Universitas Binawan, Jakarta 13630, Indonesia*
[3]*Department of Medical Pharmacy, Faculty of Medicine, Universitas Indonesia, Jakarta 10430, Indonesia*
[4]*Department of Neurology, Faculty of Medicine, Universitas Indonesia, Jakarta 10430, Indonesia*
[5]*Department of Chemistry, Faculty of Science, Universitas Brawijaya, Malang 65145, Indonesia*

([*]Corresponding author's e-mail: erni.hernawati@ui.ac.id)

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Abstract

Insulin resistance in the brain contributes to metabolic and neurodegenerative disorders. Existing ones have a limited efficacy and a long-term safety profile for traditional therapy, mainly represented by metformin. Hence, there is an urgent necessity for a safe alternative using plant-based medications. One such candidate, *Tinospora crispa* (*T. crispa*), has shown antidiabetic properties, but there is no scientific study focusing on its effectiveness in alleviating brain insulin resistance. This study aims to evaluate the potential of *T. crispa* for brain insulin resistance via modulation of the PI3K/Akt pathway. Six-week-old Wistar rats were randomly assigned to seven different groups for the study. These included a normal control group (N), a high-fat high-fructose group (HFHF), a group treated with metformin (MET), groups receiving *T. crispa* extract at doses of 200 mg/kgBW (TC200) and 400 mg/kgBW (TC400) combinations of *T. crispa* with metformin (TC200MET, TC400MET). The rats were fed a high-fat, high-fructose (HFHF) diet for 16 weeks, with treatments beginning at week 8 after confirming insulin resistance. Body weight, glucose and insulin levels were measured at week 0, 8 and 16. Lipid profiles were assessed using colorimetry, whilebrain PI3K/Akt expressions were measured using qRT-PCR. Both were conducted at the end of the study. Results shows that A 16-week HFHF diet did not affect body weight or induce obesity in mice but led to insulin resistance, elevated fasting blood glucose and altered lipid profiles. Treatment with *T. crispa* extract or its combination with metformin improved insulin resistance, glucose homeostasis and lipid levels. Notably, increased expression of IRS, PI3K and AKT was observed only in the TC400 and TC400MET groups. These findings suggest that *T. crispa* extract exhibits neuroprotective effects as an insulin sensitizer via the PI3K/Akt pathway, both as a monotherapy and in combination with metformin.

Keywords: Insulin resistance, *Tinospora crispa*, PI3K, AKT, HFHF

Introduction

Insulin resistance can arise from various factors, including genetic predisposition, physical inactivity, obesity, chronic inflammation and unhealthy dietary habit [1-4]. Currently, poor diet has become a major concern due to the increasing public tendency to consume fast food and processed beverages. The easy accessibility and widespread availability of these products contribute significantly to their high consumption. Diets rich in carbohydrates and fats have been linked to several metabolic disturbances, particularly insulin resistance [5]. Moreover, many commercially available packaged drinks contain high levels of fructose, used as an artificial sweetener and flavor enhancer. Excessive fructose intake promotes triglyceride and cholesterol accumulation due to its lipogenic effects, thereby further decreasing insulin

sensitivity and contributing to insulin resistance and glucose intolerance [5]. In addition to affecting peripheral tissues, insulin resistance can also occur in the brain—a condition referred to as cerebral insulin resistance. This occurs when insulin receptor (IR) signaling in neurons is disrupted, preventing insulin from effectively binding to its receptors on the neuronal cell membrane. Consequently, impaired IR signaling fails to activate the phosphatidylinositol 3-kinase (PI3K)–protein kinase B (Akt) pathway, which is essential for neuronal survival and function.

Managing insulin resistance primarily involves lifestyle modifications, including adopting a healthy diet, increasing physical activity and achieving weight loss when necessary. In certain cases, pharmacological intervention is required to improve insulin sensitivity and maintain blood glucose levels within the normal range. Metformin, a first-line treatment for type 2 diabetes mellitus (T2DM), remains the most widely used antidiabetic agent globally [6,7]. Recent studies have shown that metformin also exerts effects on brain insulin signaling by modulating insulin receptors, insulin receptor substrate-1 (IRS-1) and the PI3K/Akt pathway [8]. Additionally, metformin has been reported to alleviate oxidative stress and reduce cerebral insulin resistance, supporting its role as an insulin-sensitizing agent. However, long-term administration or excessive dosing of metformin may lead to several adverse effects, including diarrhea [9], weight loss [10], impaired renal function [11], hepatic injury [12] and the most commonly reported complaint, gastrointestinal disturbances [13,14]. Therefore, safer and more natural therapeutic alternatives are needed.

Several natural compounds have been explored to mitigate the effects of insulin resistance, one of which is *T. crispa*. This plant possesses notable antioxidant and anti-inflammatory properties, which are believed to contribute to improved insulin sensitivity. *T. crispa* extract has been extensively studied and utilized in experimental models of diabetes mellitus (DM), demonstrating significant antidiabetic effects [15,16]. Prior studies have identified borapetoside, a key bioactive compound in *T. crispa*, as effective in alleviating hyperglycemia, insulin resistance, hepatic steatosis and hyperlipidemia in type 2 diabetes mellitus (T2DM) animal models [17]. More recent research has highlighted the activity of tinocorside A, another active compound in *T. crispa*, which showed strong binding affinity to the PI3K protein—an essential component of the insulin receptor signaling pathway—through molecular docking analysis [18]. These findings support the role of *T. crispa* as an insulin sensitizer, capable of forming stable ligand–protein conformations and hydrogen bonds.

Previous studies have established the antidiabetic and lipid-lowering properties of *Tinospora crispa*, with a primary focus on peripheral insulin sensitivity. However, no studies have explored its potential to target brain insulin resistance or its role in regulating the PI3K/Akt signaling pathway in the central nervous system. This study addresses this critical gap by demonstrating that *T. crispa* not only improves peripheral metabolic parameters but also restores brain insulin signaling impaired by an HFHF diet. These findings provide novel evidence for the neuroprotective potential of *T. crispa*, a plant-based option for addressing both metabolic and neurodegenerative disorders.

Materials and methods

Extraction procedure

The stem simplicia of *T. crispa* was obtained from the Center for Research and Development of Medicinal Plants and Traditional Medicine, Karanganyar, Indonesia and identified as *Tinospora crispa* (L.) Hook.f. & Thomson by the Tawangmangu Traditional Health Service Unit, Ministry of Health, Indonesia. The dried stems (3 kg total) were ground into powder and extracted by maceration with 70% ethanol (Merck, 107017, Darmstadt, Germany) at a ratio of 1:3 (w/v). Each 500 g batch was macerated at room temperature for 24 h and the residue was re-extracted twice with the same solvent and ratio for 48 and 72 h, respectively. All filtrates were combined and evaporated at ± 50 °C using a rotary evaporator (Eyela, 281208, Tokyo, Japan) to obtain a thick extract. The concentrated extract was then subjected to organoleptic observation, where its sensory characteristics were assessed. Subsequently, the yield obtained from the extraction process was calculated and, followed by determination of the percentage of moisture content, ash content, acid-insoluble ash content, water-soluble extractive matter and ethanol soluble extractive matter. A phytochemical screening to ascertain the presence of different bioactive compounds as well as

heavy metal assessment for safety and quality control is also a pre-condition.

Animal and experimental design

This study used male Wistar rats (*Rattus norvegicus*) aged 6 - 8 weeks, weighing 150 - 200 g, in healthy condition (active movement). The animals were obtained from CV. Dunia Kaca, Kemuning, Central Java. Ethical approval was granted by the Health Research Ethics Committee of Faculty of Medicine, University of Indonesia (No. KET-1752/UN2.F1/ETIK/PPM.00.02/2023). Each group consisted of 4 rats, with 1 additional rat per group added to account for a potential 10% dropout, resulting in 5 rats per group. Rats that showed signs of illness during observation were excluded from the study. The animals were housed at the Animal Research Facility, IMERI, University of Indonesia, in polypropylene cages (50×40×20 cm) with rust-resistant metal lids and wood shavings as bedding. Each cage housed 2 - 3 rats and was maintained in a controlled environment: 12-hour light/dark cycle, 21 - 22 °C temperature and 60 - 70% humidity. Food and water were provided ad libitum.

Male Wistar rat aged 6 - 8 weeks, weight of 150 - 200 g in active movement healthy state was used as subject. Following seven-day acclimatization period, the rats were randomly divided into two groups: A control group (n = 5), which was fed a standard rodent diet with water ad libitum for 16 weeks and an HFHF group (n = 30) that was provided with the high fat and high fructose diet for 16 weeks. The high-fat diet consisted of a regular diet supplemented with quail egg yolk, making up 10% of the total weight. The daily dose was 10 g of feed. The high-fructose diet involved administering 825 mg of 55% fructose in 1.5 mL via intragastric gavage twice daily during feeding, resulting in a total daily dose of 1,650 mg [19].

After completing the 8-week HFHF diet regimen, rats were injected intraperitoneally with a single dose of streptozotocin (STZ, 22 mg/kg BW [20]) on the following day (day 58) under fasting conditions. Following confirmation of insulin resistance, HFHF-diet rats were randomly allocated to six treatment groups: HFHF (control), MET (metformin), TC200, TC400, TC200MET and TC400MET, with each group containing five rats (n = 5). Subsequently, rats in each group received their designated oral treatments—metformin (100 mg/kg/day [21-23]), *T. crispa* extract at a doses of 200 or 400 mg/kg/day)[24,25] or a combination of the extract and metformin—while continuing on a high-fat diet and receiving a once-daily oral fructose administration for the next 8 weeks. The animals were served daily for the status of health and any dead animals were excluded from the study.

Assessment of fasting blood glucose, fasting insulin and HOMA-IR Levels

Fasting blood glucose (FBG), fasting insulin (FI) and HOMA-IR were measured at three time points: Day 0 (baseline, post-acclimatization), day 59 (after HFHF diet and STZ injection) and day 114 (post-8-week therapy). FBG was measured using a GlucoDr. Auto A glucometer (Allmedicus, AGM-4000, Anyang-si, South Korea). FI was assessed using a Rat Insulin ELISA kit (Elabscience, E-EL-R3034, Houston, TX, USA) and read with a Varioskan™ Flash Multimode Reader (Thermo Fisher Scientific, 5250030, Waltham, USA). HOMA-IR was calculated using the standard formula [26,27]:

$$HOMA\text{-}IR = \frac{FBG\left(\frac{mg}{dL}\right) \times FI\left(\frac{ng}{mL}\right) \times 26}{405}$$

Lipid profile measurement using enzymatic colorimetric method

Lipid profile assessment was performed once after the completion of therapy on serum samples collected after overnight fasting. Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and triglyceride (TG) levels were evaluated with enzymatic colorimetric methods with commercial kits (Elabscience, E-BC-K221; E-BC-K109-M; E-BC-K261-M, Wuhan, China) following the manufacturer's protocols and quantified with a microplate reader Varioskan™ Flash Multimode Reader (Thermo Fisher Scientific, 5250030, Waltham, USA). Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation:

$$LDL\text{–}C = TC - HDL\text{–}C - \frac{TG}{5}$$

Quantitative RT-PCR analysis

At the end of the study, the rats were sacrificed and brain tissues from the cortex and hippocampus were collected and stored at −80 °C. Approximately 10 mg of tissue was homogenized in 500 µL Buffer RL (Vazyme, RC112-01, China) using an electric homogenizer. Total RNA was extracted using the FastPure Cell/Tissue Total RNA Isolation Kit (Vazyme, RC112-01, China) and reverse transcribed into cDNA using the HiScript® III RT SuperMix for qPCR (+gDNA wiper) kit (Vazyme, R323, China). RNA and cDNA purity and concentration were assessed at 260/280 nm using the Varioskan™ Flash Multimode Reader (Thermo Fisher Scientific, 5250030, Waltham, USA). Quantitative PCR was performed with the SensiFAST™ SYBR® No-ROX Kit (Bioline, BIO-98005, Meridian Bioscience, UK). The primer sequences (5′–3′) used for qPCR are listed in **Table 1**.

Table 1 List of primer sequences for target genes.

No	Gen	Forward Primer (5′–3′)	Reverse Primer (5′–3′)
1	*IRS1*	GAG TTG AGT TGG GCA GAG TAG	CAT GTA ATC ACC ACG GCT ATT TG
2	*PIK3CA*	GAC AAG AAC AAG GGC GAG ATA	CGG TCT CCA ATT CCC AAG ATA A
3	*AKT2*	GGA GGA TGC CAT GGA TTA CAA	CTT GCC GAG GAG TTT GAG ATA A
4	*ACTB*	AGC CAT GTA CGT AGC CAT CC	AGC CAT GTA CGT AGC CAT CC

Thermal cycling conditions included initial polymerase activation at 95 °C for 2 min, followed by 40 cycles of denaturation at 95 °C for 5 s, annealing at 60 °C for 10 s and extension at 72 °C for 15 s. A dissociation (melting curve) stage was conducted at 72 °C for 7 min, 59 °C for 60 s and 95 °C for 15 s. Amplification and data analysis were performed using a Real-Time Quantitative Thermal Cycler (Vazyme, Nanjing, China) and relative gene expression levels were calculated using the ΔΔCT method.

Phospho-Akt ELISA

Brain tissue, harvested at the end of the experiment and preserved at −80 °C, was used for this analysis. Phosphorylated Akt (Ser473) levels were measured using a commercial ELISA kit (BT Lab, E2452Ra, Beijing, China) following the manufacturer's protocol. Briefly, standards were prepared by serial dilution and 40 µL of sample with 10 µL biotinylated antibody and 50 µL streptavidin-HRP were added to the wells. After incubation for 60 min at 37 °C, wells were washed five times with wash buffer. Substrate solutions A and B (50 µL each) were added and incubated for 10 min at 37 °C in the dark. The reaction was stopped with 50 µL stop solution and absorbance was read at 450 nm using a microplate reader. Concentrations were determined from a standard curve.

Statistical analysis

Statistical analyses were performed using GraphPad Prism version 10.4.0 and data are expressed as mean ± SD. Data normality and variance homogeneity were evaluated with the Shapiro–Wilk and Levene tests, respectively, to guide the selection of statistical methods. For parametric data, one-way ANOVA was applied, followed by Tukey's or Least Significant Difference (LSD) post hoc comparisons. For non-parametric data, the Kruskal–Wallis test was employed, with subsequent Dunn's tests. Statistical significance was set at $p\text{-value} < 0.05$.

Results and discussion

Extraction results and quality evaluation of *T. crispa* extract

T. crispa extract appeared as a thick, greenish-brown substance with a characteristic odor (**Table 2**). Extraction using ethanol as the solvent yielded 17.84%, with a moisture content of 23.71%. The physicochemical characterization revealed a total ash content of 11.89%, acid-insoluble ash of 0.05%, water-soluble extractive content of 28.01% and ethanol-

soluble extractive content of 28.89%. Phytochemical screening indicated the presence of secondary metabolites, including flavonoids, tannins, saponins, steroids, triterpenoids and glycosides. Heavy metal analysis confirmed the absence of lead (Pb) and cadmium (Cd) in the extract.

Table 2 Evaluation results of *T. crispa* ethanol extract.

Parameter	Result
Organoleptic	
Form	Thick
Odor	Characteristic
Color	Brownish green
Yield	17.84%
Ash Content	11.89%
Moisture Content	23.71%
Acid-insoluble Ash Content	0.05%
Water-soluble Extractive Content	28.01%
Ethanol-soluble Extractive Content	28.89%
Phytochemical Screening	
Flavonoids	(+)
Alkaloids	(−)
Tannins	(+)
Saponins	(+)
Steroids	(+)
Triterpenoids	(+)
Glycosides	(+)
Heavy Metals	
Lead (Pb)	Not detected
Cadmium (Cd)	Not detected

HFHF diet administration induces hyperglycemia and insulin resistance without causing obesity

At baseline, the body weights of the standard diet (N) and HFHF diet groups were 185.6 ± 16.30 g and 181 ± 18.86 g, respectively. After 8 weeks, both groups showed a significant increase, reaching 237 ± 22.12 g and 238 ± 26.28 g. Correspondingly, body length also increased from 19.23 ± 0.49 cm (N) and 19.01 ± 0.41 cm (HFHF) to 21.94 ± 0.48 cm and 21.73 ± 0.26 cm post HFHF+STZ induction. Lee index values at baseline were 281.22 ± 6.29 (N) and 280.4 ± 7.24 (HFHF), which increased to 298.92 ± 6.02 and 299.58 ± 7.27 after 8 weeks (**Table 3**). However, there were no statistically

significant differences in body weight, body length, or Lee index between the N and HFHF groups at week 8.

FBD levels increased significantly in the HFHF group after 8 weeks of diet induction, while no significant change was observed in the N group. In the N group, glucose levels before and after induction were 93.50 ± 3.11 mg/dL and 91.75 ± 6.34 mg/dL, respectively. In contrast, the HFHF group showed a significant rise from 89.0 ± 1.41 mg/dL to 159.0 ± 10.17 mg/dL. The increase in blood glucose levels in the HFHF group was accompanied by a rise in fasting insulin levels after 8 weeks. In the N group, fasting insulin decreased from 0.34 ± 0.04 to 0.18 ± 0.02 ng/mL, remaining within the normal range (< 0.6 ng/mL) [26,27]. Conversely, the HFHF group showed a significant increase in insulin levels from 0.32 ± 0.04 to 0.70 ± 0.10 ng/mL. This change was consistent with the HOMA-IR values, which increased from 1.56 ± 0.22 to 6.78 ± 1.42 in the HFHF group, while values in the N group decreased from 1.86 ± 0.14 to 0.99 ± 0.11 (**Table 3**). A HOMA-IR value > 2.6 [28,29] indicates insulin resistance, confirming successful induction of the insulin-resistant model.

Table 3 Characteristics of experimental animals at week 0 and week 8.

Variabel	Week 0		Week 8	
	N	HFHF	N	HFHF
Body weight (gram)	185.6 ± 16.30[a]	181.0 ± 18.86[a]	237 ± 22.12[b]	238 ± 26.28[b]
Body length (cm)	19.23 ± 0.49[a]	19.00 ± 0.4[a]	21.94 ± 0.48[b]	21.73 ± 0.26[b]
Lee index	281.22 ± 6.29[a]	280.41 ± 7.24[a]	298.92 ± 6.02[b]	299.58 ± 7.27[b]
FBG (mg/dL)	93.50 ± 3.1[a]	89.00 ± 1.41[b]	91.75 ± 6.34[b]	159.00 ± 10.17[c]
FI (ng/mL)	0.34 ± 0.04[a]	0.32 ± 0.04[a]	0.18 ± 0.02[b]	0.70 ± 0.01[c]
HOMA-IR (mg/dL x µU/mL)	1.86 ± 0.14[a]	1.56 ± 0.22[a]	0.99 ± 0.11[a]	6.78 ± 1.42[b]

A high-fat high-fructose (HFHF) diet combined with a single intraperitoneal dose of streptozotocin (STZ) has been widely recognized as an effective model for inducing insulin resistance and metabolic dysfunction in experimental animals. In this study, although the rats demonstrated normal weight gain and did not develop obesity, significant disruptions in glucose homeostasis were observed, as evidenced by elevated fasting blood glucose, fasting insulin, and HOMA-IR index. These findings suggest that systemic insulin resistance can develop independently of obesity, supporting previous evidence that non-adipose tissues such as the liver and brain play a critical role in the pathogenesis of insulin resistance [30,31]

Effect of *T. crispa* extract on weight gain and lee index

At the end of the study, the mean body weights (g) of the rats in groups N, HFHF, MET, TC200, TC400, TC200MET and TC400MET were 267 ± 6.63, 308 ± 8.00, 228 ± 44.78, 266.5 ± 3.78, 252 ± 25.03, 267.5 ± 18.43 and 271.5 ± 8.00, respectively. As shown in **Figure 1(A)**, the HFHF group exhibited increased body weight following HFHF induction, although the increase was not statistically significant compared to the N group. Treatment with *T. crispa* extract (TC200, TC400, TC200MET, TC400MET) led to a reduction in body weight; however, the differences were not significant compared to the HFHF group. A significant decrease in body weight was observed only in the MET group. Body length did not differ significantly among groups, with mean values (cm) for the N, HFHF, MET, TC200, TC400, TC200MET, and TC400MET groups recorded as 22.75 ± 0.20, 22.60 ± 0.11, 21.78 ± 0.86, 22.40 ± 0.25, 22.40 ± 0.27, 22.33 ± 0.34, and 22.55 ± 0.24, respectively (**Figure 1(B)**). The Lee index values for the N, HFHF, MET, TC200, TC400, TC200MET, and TC400MET groups were 281.6 ± 6.43, 298.8 ± 2.13,

285.7 ± 9.59, 289.3 ± 4.76, 281.5 ± 10.67, 282.6 ± 8.98 and 281.6 ± 5.49, respectively. Statistical analysis indicated no significant differences in Lee index values among the groups (**Figure 1(C)**). These data indicate that all groups showed both body weight and length gain throughout the study period.

The *T. crispa* extract did not show any significant impact on body weight, suggesting that it may not have a direct effect on weight regulation. On the other hand, metformin led to a marked decrease in body weight, which is consistent with its established role in promoting weight loss in diabetic and insulin-resistant conditions. These results further emphasize the distinct effects of *T. crispa* and metformin in weight management [32-34].

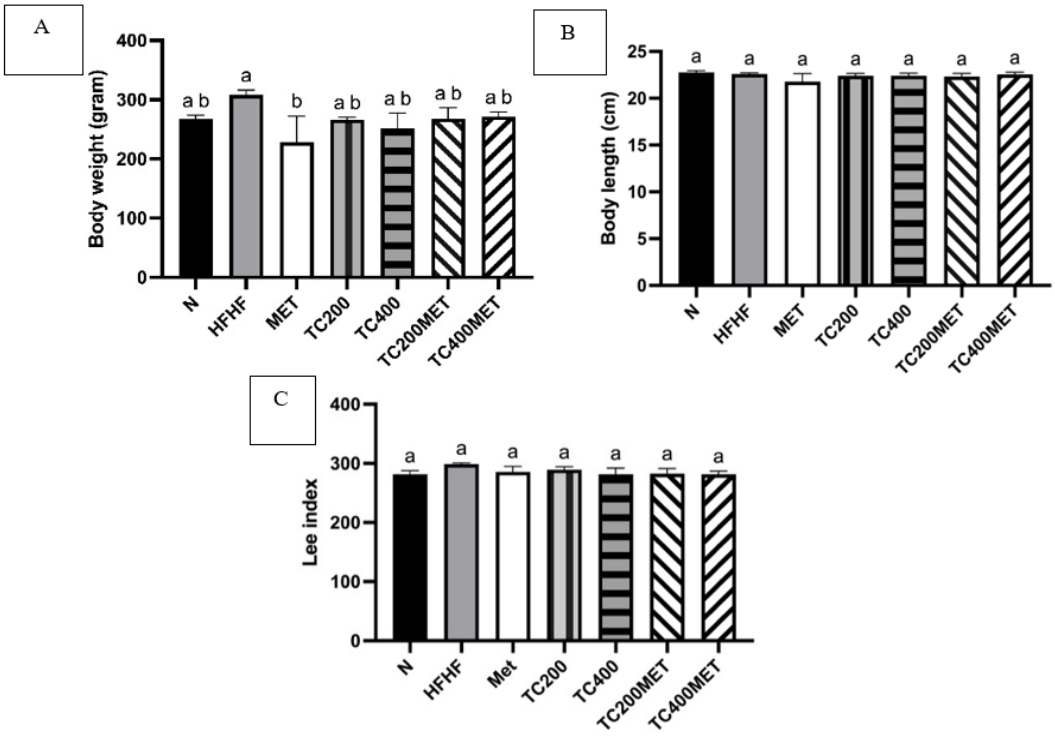

Figure 1 Graphs of body weight (A), weight gain (B) and Lee index (C) at the end of the study following *T. crispa* extract administration. Data are presented as mean ± SD. Data are presented as mean ± SD. Different legend letters (a, b) denote statistical among the groups (*p-value* ≤ 0.05).

***T. crispa* extract ameliorates FBG, FI and HOMA-IR**

As shown in **Figure 2(A)**, by the end of study, the mean FBG level in the HFHF group was 165.8 ± 6.23 mg/dL, which was significantly higher than that of the N group (81.50 ± 5.68 mg/dL). Significant reductions in fasting blood glucose were observed in the Met, TC400, TC200MET and TC400MET groups, with mean values of 95.25 ± 10.87, 118.5 ± 23.36, 113.0 ± 7.43 and 96.25 ± 10.59 mg/dL, respectively, compared to the HFHF group.

Consistent with the fasting blood glucose results, fasting insulin levels (**Figure 2(B)**) significantly decreased in all treatment groups compared to the HFHF group. The mean fasting insulin levels in the Met, TC200, TC400, TC200MET, and TC400MET groups were 0.42 ± 0.04, 0.46 ± 0.02, 0.35 ± 0.07 and 0.34 ± 0.04 ng/mL, respectively—values that were comparable to those of the N group (0.33 ± 0.04 ng/mL) and significantly lower than the HFHF group (0.92 ± 0.01 ng/mL). These findings indicate a significant increase in insulin levels following HFHF induction, which was attenuated by the subsequent treatments.

The HOMA-IR values of the N and HFHF groups were significantly different, with mean values of 1.42 ± 0.16 and 4.27 ± 0.35, respectively (**Figure 2(C)**). Following 8 weeks of treatment, the HOMA-IR values in the Met, TC200, TC400, TC200MET and TC400MET groups significantly decreased compared to the HFHF group, with average values of 2.44 ± 0.43,

2.95 ± 0.21, 2.15 ± 0.68, 2.47 ± 0.18 and 2.17 ± 0.45, respectively. These findings indicate that the HFHF+STZ diet induced a marked increase in insulin resistance, as reflected by the elevated HOMA-IR in the HFHF group relative to the N group. Treatment with *T. crispa* extract, either alone or in combination with metformin, effectively ameliorated insulin resistance.

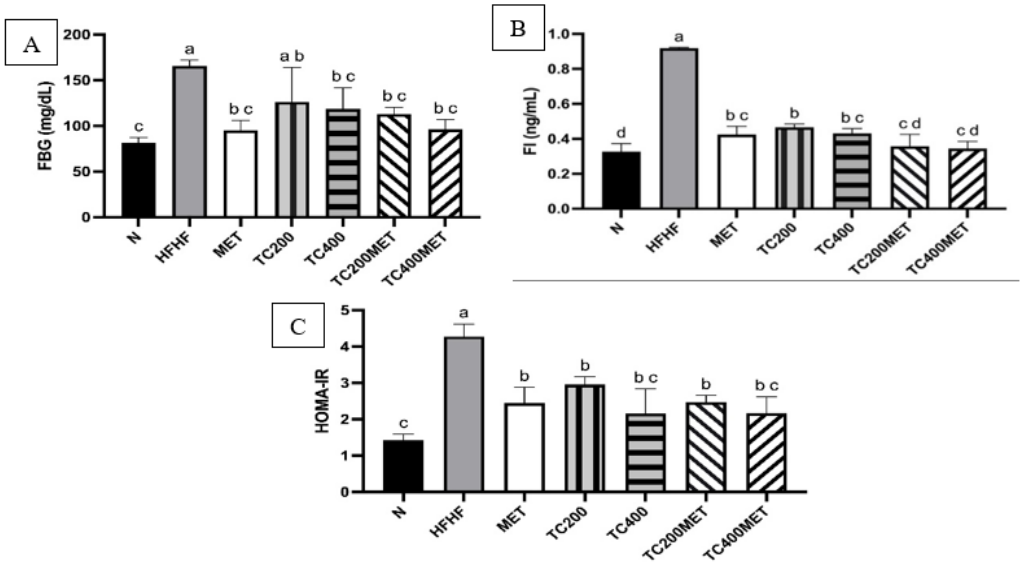

Figure 2 Graphs of fasting blood glucose (A), fasting insulin (B) and HOMA-IR (C) at the end of the study after administration of *T. crispa* extract. Data are presented as mean ± SD. Data are presented as mean ± SD. Different legend letters (a, b, c, d) denote statistical among the groups (p-value ≤ 0.05). The presence of duplicate letters indicates no significant difference between those groups.

Therapeutic administration of *T. crispa* extract at doses of 200 and 400 mg/kgBW, either as monotherapy or in combination with metformin, significantly improved fasting blood glucose and HOMA-IR levels. These results align with previous *in vivo* studies reporting that bioactive constituents of *T. crispa*, such as borapetoside and tinocorside A, enhance insulin sensitivity via activation of the PI3K/Akt insulin signaling pathway [15-18]. Moreover, its combination with metformin demonstrated a potential synergistic effect, offering a promising and safer alternative for long-term management of insulin resistance.

Effect of *T. crispa* extract on lipid profile

The cholesterol profiles across the seven groups exhibited similar trends and were consistent with the observed levels of total cholesterol (TC), triglycerides (TG) and low-density lipoprotein cholesterol (LDL-C). A marked increase in TC, TG and LDL-C levels was observed in the HFHF group compared to the normal (N) group at the end of the study, indicating dyslipidemia induced by the HFHF diet. In contrast, all therapy groups showed significantly reduced levels of these parameters compared to the HFHF group. The most pronounced reduction was observed in the group treated with *T. crispa* extract at a dose of 400 mg/kgBW (**Figures 3(A) - 3(C)**). Consistent with these findings, high-density lipoprotein cholesterol (HDL-C) levels were significantly lower in the HFHF group than in the N group. Treatment with the extract alone or in combination with metformin significantly increased HDL-C levels compared to the HFHF group (**Figure 3(D)**).

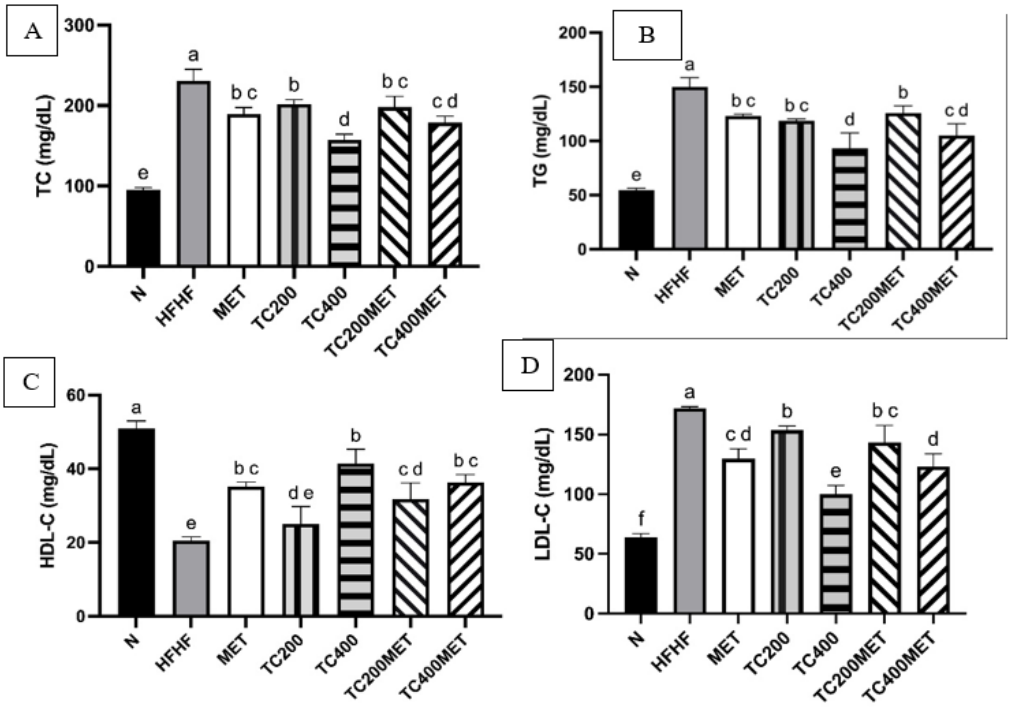

Figure 3 Graphs of lipid profile. Level of TC (A), TG (B), LDL-C (C) and HDL-C (D). Following treatment with *T. crispa* extract. Data are presented as mean ± SD. Data are presented as mean ± SD. Different legend letters (a, b, c, d) denote statistical among the groups (*p-value* ≤ 0.05). The presence of duplicate letters indicates no significant difference between those groups.

T. crispa extract not only improves insulin resistance but also significantly enhances lipid profiles by reducing triglycerides, total cholesterol and LDL levels, while increasing HDL. These findings suggest that *T. crispa* possesses important hypolipidemic properties, potentially lowering the risk of cardiovascular complications commonly associated with metabolic syndrome and insulin resistance. This effect is attributed to its bioactive constituents—such as alkaloids, flavonoids and diterpenoids—which are known to inhibit lipogenesis and promote fatty acid oxidation. These results are in line with previous studies, that bioactive compounds present in *T. crispa* extract reduces serum lipid levels and improves metabolic profiles in diabetic rats [17,35-37]. Considering that hyperlipidemia plays a pivotal role in the pathogenesis of insulin resistance by promoting lipotoxicity and disrupting insulin signaling pathways, the capacity of *T. crispa* to lower triglyceride levels and improve overall lipid profiles may confer dual therapeutic effects—ameliorating dyslipidemia while concurrently alleviating lipid-induced impairments in insulin signaling mechanisms [38-40].

***T. crispa* extract modulates PI3K/AKT signaling pathway in the rat brain**

One of the key proteins that works as a mediator when insulin connects to its receptor on the cell's surface is IRS1. Once insulin binds, it triggers the phosphorylation of IRS1, setting off a cascade of signals within the cell, including the PI3K/AKT pathway. This process plays a crucial role in regulating various cellular functions, including the regulation of glucose metabolism. The analysis of IRS1 gene expression revealed a significant decrease in IRS1 mRNA levels in the HFHF group compared to the N group. Following treatment, relative mRNA expression levels in the Met, TC400, TC200Met and TC400Met groups increased significantly compared to the HFHF group, as illustrated in **Figure 4(A)** The most substantial increase was observed in the TC400Met group, where the relative mRNA expression increased by 0.84-fold, approaching the expression level observed in the N group. The relative mRNA expression of PIK3CA, a crucial gene that codes for the catalytic subunit of PI3K, showed a significant reduction to 0.048-fold compared to the N

group post-administration of HFHF induction (**Figure 4(B)**). After treatment, PIK3CA expression significantly increased in groups receiving metformin alone, *T. crispa* extract at a dose of 400 mg/kgBW, or its combination with metformin. A similar pattern was observed in the relative mRNA expression of the AKT2 gene (**Figure 4(C)),** with elevated expression in groups treated with metformin, *T. crispa* extract (400 mg/kgBW) and both combination therapy groups. However, only the TC400 and TC400MET groups demonstrated a statistically significant increase in AKT2 expression compared to the HFHF group. At the end of the study, the HFHF group showed a marked decrease in p-AKT concentration levels compared to the N group (**Figure 4(D)**), indicating impaired activation of the insulin signaling pathway. Notably, only the TC400 treatment group showed a significant increase in p-AKT levels relative to the HFHF group. These levels approached those observed in the N group, suggesting that *T. crispa* extract may enhance AKT phosphorylation and partially restore insulin.

Notably, the 400 mg/kgBW dose of *T. crispa* was more effective in restoring insulin sensitivity than the 200 mg/kgBW dose, as shown by consistent improvements in gene expression across key nodes of the insulin signaling cascade. Therapeutic intervention significantly improved the mRNA expression of IRS1, PIK3CA and AKT2, particularly in the groups treated with metformin (MET), *T. crispa* extract at 400 mg/kgBW (TC400) and their combination (TC400MET), compared to the HFHF group (*p-value* < 0.05). These findings suggest that high-dose *T. crispa* extract effectively reactivates the PI3K/AKT insulin signaling pathway disrupted by the HFHF diet. This reactivation is evident not only at the downstream level (PIK3CA and AKT2), but also through enhanced upstream regulation via IRS1 expression [8], which plays a critical role in initiating insulin signal transduction. The PI3K/AKT pathway activation contributes to reduced oxidative stress and improved glucose transport in the brain, which are critical components in addressing cerebral insulin resistance. Impairments in this signaling pathway not only affect insulin metabolism, but also are closely linked to the pathogenesis of neurodegenerative and cognitive decline [30,31]. Overall, these results suggest that *T. crispa* has the same or even better potential in certain areas compared to metformin as a candidate for plant-based therapy in modulating insulin resistance improvement through the PI3K/AKT pathway.

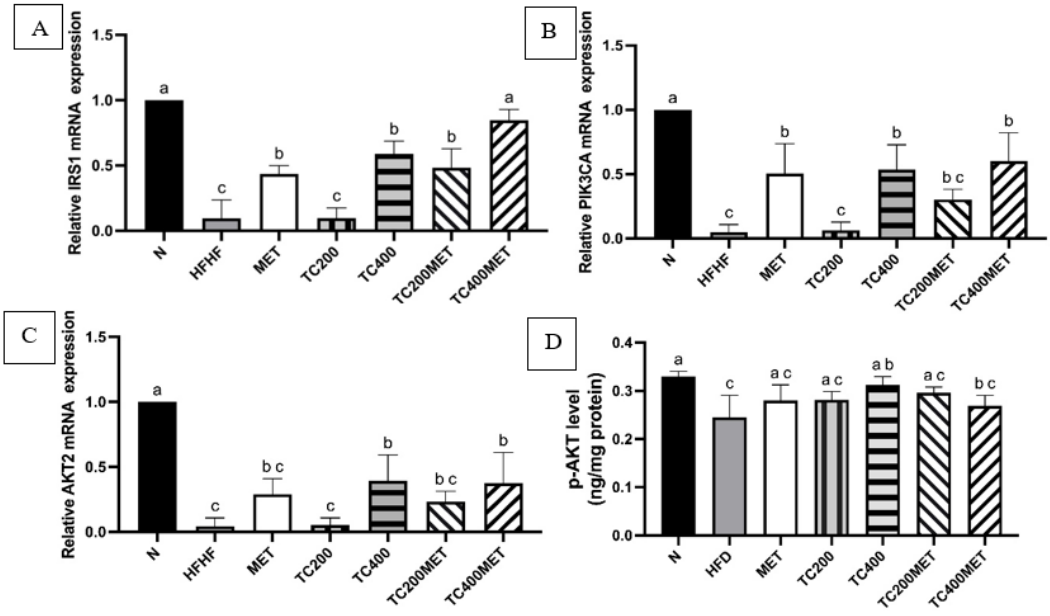

Figure 4 Graphs of related gene or protein to PI3K/AKT Pathway. Expression of relative IRS1 (A), PIK3CA (B), AKT2 (C) mRNA and protein level of p-AKT (D) post-administration of *T. crispa* extract. Data are presented as mean ± SD. Data are presented as mean ± SD. Different legend letters (a, b, c, d) denote statistical among the groups (*p-value* ≤ 0.05). The presence of duplicate letters indicates no significant difference between those groups.

Interestingly, gene expression results in the group receiving the combination of extract and metformin (TC400MET) showed no significant differences compared to the group receiving only the extract at a dose of 400 mg/kgBW, indicating that *T. crispa* at this dose is quite effective as a monotherapy or adjunct therapy with metformin. These findings support the candidacy of *T. crispa* as an alternative natural insulin sensitizer with comparable efficacy to conventional agents and potentially better safety for long-term use. However, the possibility of synergistic interactions between *T. crispa* and metformin cannot be excluded. Further research is warranted to explore their potential interaction and to optimize combination strategies.

Previous studies have established the antidiabetic and lipid-lowering properties of *Tinospora crispa*, primarily focusing on peripheral insulin sensitivity. However, to date, no research has specifically examined its effects on brain insulin resistance or the modulation of the PI3K/Akt signaling pathway within the central nervous system. This study addresses this critical gap by demonstrating that *T. crispa* not only improves peripheral metabolic parameters but also restores brain insulin signaling impaired by a high-fat, high-fructose diet. These findings provide novel evidence for the neuroprotective potential of *T. crispa*, offering new insights into plant-based strategies for combating metabolic and neurodegenerative disorders.

These findings could form the basis for developing phytopharmaceutical formulations or herbal supplements targeting the PI3K/Akt pathway to prevent or treat brain metabolic disorders. With further validation through clinical trials, Tinospora crispa has the potential to be integrated into environmentally friendly and affordable programs for the prevention of degenerative diseases. Furthermore, this approach could encourage innovation in more efficient and environmentally friendly active ingredient extraction technologies, expanding commercialization opportunities and collaboration between the research sector, the herbal pharmaceutical industry and the agricultural community

Conclusions

The present study demonstrates that administration of *T. crispa* extract, particularly at a dose of 400 mg/kgBW, significantly improves insulin sensitivity and glucose homeostasis in HFHF-STZ–induced insulin-resistant rats. This therapeutic effect is evidenced by decreased fasting blood glucose, insulin levels and HOMA-IR index, as well as improved lipid profiles. Furthermore, the extract effectively restores the expression of key insulin signaling genes—IRS1, PIK3CA and AKT2—indicating reactivation of the PI3K/Akt pathway. Notably, the combination of *T. crispa* with metformin did not show additive effects beyond the extract alone at high doses, suggesting *T. crispa* may serve as an effective monotherapy. These findings support the potential of *T. crispa* as a natural insulin sensitizer and a promising phytotherapeutic agent for managing insulin resistance and its associated metabolic complications. Further studies are warranted to explore its long-term safety, synergistic mechanisms with standard drugs and its applicability in clinical settings.

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Declaration of generative AI in scientific writing

During the preparation of this manuscript, the author utilized Grammarly to improve grammar, sentence structure, and the readability and language of a manuscript. Following the application of this tool/service, the author thoroughly reviewed and edited the content as necessary and took full responsibility for the final published version.

CRediT author statement

Kartika Rahma: Conceptualization; Methodology; Formal analysis; Formal analysis; Data Curation; Writing - Original Draft; Visualization. **Erni Hernawati Purwaningsih:** Conceptualization; Methodology; Validation; Resources; Data Curation; Supervision; Project administration; Funding

acquisition. **Yetty Ramli:** Methodology; Validation; Writing - Review & Editing; Supervision. **Aulanniam Aulanniam:** Methodology; Validation; Writing - Review & Editing; Supervision

References

[1] YK Chen, TT Liu, FKF Teia and MZ Xie. Exploring the underlying mechanisms of obesity and diabetes and the potential of traditional chinese medicine: An overview of the literature. *Frontiers in Endocrinology* 2023; **14**, 1218880.

[2] SK Ahmed and RA Mohammed. Obesity: Prevalence, causes, consequences, management, preventive strategies and future research directions. *Metabolism Open* 2025; **27**, 100375.

[3] MS Ellulu and H Samouda. Clinical and biological risk factors associated with inflammation in patients with type 2 diabetes mellitus. *BMC Endocrine Disorders* 2022; **22(1)**, 16.

[4] P Arya. Risk factors of diabetes. *Journal of Diabetes Medication & Care* 2023; **6(3)**, 61-66.

[5] R Hegazi, M El-Gamal, N Abdel-Hady and O Hamdy. Epidemiology of and risk factors for type 2 diabetes in egypt. *Annals of Global Health* 2015; **81(6)**, 814-820.

[6] CJ Bailey. Metformin: Therapeutic profile in the treatment of type 2 diabetes. *Diabetes, Obesity & Metabolism* 2024; **26(S3)**, 3-19.

[7] CT Roberts, N Raabe, L Wiegand, AK Shahib and M Rastegar. Diverse applications of the anti-diabetic drug metformin in treating human disease. *Pharmaceuticals* 2024; **17(12)**, 1601.

[8] M Zheng and P Wang. Role of insulin receptor substance-1 modulating PI3K/Akt insulin signaling pathway in alzheimer's disease. *3 Biotech* 2021; **11(4)**, 179.

[9] H Takemori, A Hamamoto, K Isogawa, M Ito, M Takagi, H Morino, T Miura, K Oshida and T Shibata. Mouse model of metformin-induced diarrhea. *BMJ Open Diabetes Research & Care* 2020; **8(1)**, e000898.

[10] K Subramaniam, MP Joseph and LA Babu. A Common drug causing a common side effect at an uncommon time: Metformin-induced chronic diarrhea and weight loss after years of treatment. *Clinical Diabetes : a Publication of the American Diabetes Association* 2021; **39(2)**, 237-240.

[11] T Congiu, M Alghrably, AH Emwas, L Jaremko, JI Lachowicz, M Piludu, M Piras, G Faa, G Pichiri, M Jaremko and P Coni. Undercover toxic ménage à trois of amylin, copper (II) and metformin in human embryonic kidney cells. *Pharmaceutics* 2021; **13(6)**, 830.

[12] L Sun, Y Wu, J Chen, J Zhong, F Zeng and S Wu. A turn-on optoacoustic probe for imaging metformin-induced upregulation of hepatic hydrogen sulfide and subsequent liver injury. *Theranostics* 2019; **9(1)**, 77-89.

[13] I Szymczak-Pajor, J Drzewoski, S Wenclewska and A Śliwińska. Metformin-Associated gastrointestinal adverse events are reduced by probiotics: A meta-analysis. *Pharmaceuticals* 2024; **17(7)**, 898.

[14] K Nabrdalik, M Hendel, K Irlik, H Kwiendacz1, I Łoniewski, T Bucci, U Alam, GYH Lip, J Gumprecht and K Skonieczna-Żydecka. Gastrointestinal adverse events of metformin treatment in patients with type 2 diabetes mellitus: A systematic review and meta-analysis with meta-regression of observational studies. *BMC Endocrine Disorders* 2024; **24(1)**, 206.

[15] UM Zuhri, ND Yuliana, F Fadilah, L Erlina, EH Purwaningsih and A Khatib. Exploration of the main active metabolites from Tinospora crispa (L.) Hook. f. & Thomson stem as insulin sensitizer in L6.C11 skeletal muscle cell by integrating *in vitro*, metabolomics, and molecular docking. *Journal of Ethnopharmacology* 2024; **319(P3)**, 117296.

[16] FH Arifah, AE Nugroho, A Rohman and W Sujarwo. A bibliometric analysis to preclinical studies of Tinospora Crispa (L.) Hook. F. & Thomson as an antidiabetic. *Indonesian Journal of Pharmacy.* 2023; **34(1)**, 24-35.

[17] Y Xu, , Y Niu, Y Gao, F Wang, W Qin, Y Lu, J Hu, L Peng, J Liu and W Xiong. Borapetoside E, a clerodane diterpenoid extracted from tinospora crispa, improves hyperglycemia and hyperlipidemia in high-fat-diet-induced type 2 diabetes mice. *Journal of Natural Products* 2017; **80(8)**, 2319-2327.

[18] UM Zuhri, EH Purwaningsih, F Fadilah and ND Yuliana. Network pharmacology integrated molecular dynamics reveals the bioactive

compounds and potential targets of Tinospora crispa Linn. as insulin sensitizer. *PLoS One* 2022; **17(6)**, e0251837.

[19] DGB Krisnamurti, FAG Sinuraya, TE Firsty, RW Hakim and EH Purwaningsih. Uric acid and glucose level in high fructose high cholesterol induced sprague-dawley rats after therapy with acalypha indica linn. ethanol extract. *AIP Conference Proceedings* 2019; **2193**, 030014.

[20] S Gunawan, E Munika, ET Wulandari, F Ferdinal, EH Purwaningsih, PE Wuyun, M Louisa and V Soetikno. 6-Gingerol ameliorates weight gain and insulin resistance in metabolic syndrome rats by regulating adipocytokines. *Saudi Pharmaceutical Journal* 2023; **31(3)**, 351-358.

[21] BK Koo. Metformin preserves peripheral nerve damage with comparable effects to alpha lipoic acid in streptozotocin/high-fat diet induced diabetic rats. *Diabetes & Metabolism Journal* 2021; **45(1)**, 125-126.

[22] MA Mobasher, HG El-Tantawi and KS El-Said. Metformin ameliorates oxidative stress induced by diabetes mellitus and hepatocellular carcinoma in rats. *Reports of Biochemistry & Molecular Biology* 2020; **9(1)**, 115-128.

[23] HMF Mohammad, SG Gouda, MA Eladl, AY Elkazaz, KS Elbayoumi, NE Farag, A Elshormilisy, BB Al-Ammash, A Hegazym, SM Abdelkhalig, AS Mohamed, M El-Dosoky and SA Zaitone. Metformin suppresses LRG1 and TGFβ1/ALK1-induced angiogenesis and protects against ultrastructural changes in rat diabetic nephropathy. *Biomedicine & Pharmacotherapy* 2023; **158**, 114128.

[24] S Firdausa, MM Cho, KM Maung, N Aung, N Kuzaifah S Suryawati. The blood glucose lowering effect of malaysian Tinospora crispa in rats. *Jurnal Natural* 2020; **20(1)**, 20-23.

[25] W Ahmad, I Jantan, E Kumolosasi and SNA Bukhari. Immunostimulatory effects of the standardized extract of Tinospora crispa on innate immune responses in wistar kyoto rats. *Drug Design, Development and Therapy* 2015; **9**, 296-2973.

[26] S Akieda-Asai, H Ma, W Han, J Nagata, F Yamaguchi and Y Date. Mechanism of muscle atrophy in a normal-weight rat model of type 2 diabetes established by using a soft-pellet diet. *Scientific Reports* 2024; **14(1)**, 7670.

[27] F Yamaguchi, S Akieda-Asai, E Nakamura, H Uchida, A Yamashita and Y Date. Continuous exposure of nonobese adult male rats to a soft-textured, readily absorbable diet induces insulin resistance and derangements in hepatic glucose and lipid metabolism. *The Journal of Nutrition* 2025; **155(5)**, 1387-1397.

[28] S Swaminathan, Elanthendral, TKD Edward and MJ Abirami. Diagnostic Usefulness of HOMA-β and HOMA-IR in Diabetes Mellitus - A Review. *International Journal of Pharmaceutical Research & Allied Sciences* 2019; **8(1)**, 17-24.

[29] LC Antunes, JL Elkfury, MN Jornada, KC Foletto and MC Bertoluci. Validation of HOMA-IR in a model of insulin-resistance induced by a high-fat diet in Wistar rats. *Archives of Endocrinology and Metabolism* 2016; **60(2)**, 138-142.

[30] CPD Kottaisamy, DS Raj, VP Kumar and U Sankaran. Experimental animal models for diabetes and its related complications: A review. *Laboratory Animal Research* 2021; **37(1)**, 23.

[31] R Singh, M Gholipourmalekabadi and SH Shafikhani. Animal models for type 1 and type 2 diabetes: Advantages and limitations. *Frontiers in Endocrinology* 2024; **15**, 1359685.

[32] T Chukir, L Mandel, BG Tchangc, NA Al-Mullad, LI Igelc, RB Kumarc, J Waitmanc, LJ Aronne and AP Shukla. Metformin-induced weight loss in patients with or without type 2 diabetes/prediabetes: A retrospective cohort study. *Obesity Research & Clinical Practice* 2021; **15(1)**, 64-68.

[33] NT Shurrab and ESA Arafa. Metformin: A review of its therapeutic efficacy and adverse effects. *Obesity Medicine* 2020; **17**, 100186.

[34] R Ray, AU Haq, R Chaudhry, S Burra, A Batool, MD Masmoum and I Ullah. Efficacy of metformin in weight management and glycemic control in type 2 diabetes: An updated systematic review and meta-analysis. *Journal of Advances in Medicine and Medical Research* 2024; **36(9)**, 225-239.

[35] CT Ruan, SH Lam, TC Chi, SS Lee and MJ Su. Borapetoside C from Tinospora crispa improves insulin sensitivity in diabetic mice. *Phytomedicine* 2012; **19(8-9)**, 719-724.

[36] E Haque, MS Bari, L Khandokar, J Anjum, I Jantan, V Seide and MA Haque. An updated and comprehensive review on the ethnomedicinal uses, phytochemistry, pharmacological activity and toxicological profile of Tinospora crispa (L.) Hook. f. & Thomson. *Phytochemistry reviews : Proceedings of the Phytochemical Society of Europe* 2023; **22(1)**, 211-273.

[37] ZM Shah, MKN Hasan, SM Arshad, KKA Kadir, IS Kamarazaman, Z Amom, RM Ali and DJ Arapoc. Effects of Tinospora crispa aqueous extract in regulating cholesterol metabolism in human hepatoma cancer cell line (Hep G2). *Journal of Medicinal Plants Research* 2017; **11(43)**, 673-682.

[38] V Kothari, Y Luo, T Tornabene, AM O'Neill, MW Greene, T Geetha and JR Babu. High fat diet induces brain insulin resistance and cognitive impairment in mice. *Biochim. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* 2017; **1863(2)**, 499-508.

[39] RR Castillejo, LA Ticona, A Macho-González, A Bocanegra, A Garcimartín, M Hernández-Martín, A Parfenova, S Bastida, L García-García, ME López-Oliva, FJ Sánchez-Muniz and J Benedí. Silicon-enriched meat consumption mitigates brain cortex damage associated with diabetic dyslipidemia in a late-stage type 2 diabetes mellitus rat model. *Redox Biology* 2025; **85**, 103697.

[40] J Sripetchwandee, N Chattipakorn and SC Chattipakorn. Links between obesity-induced brain insulin resistance, brain mitochondrial dysfunction and dementia. *Frontiers in Endocrinology* 2018; **9**, 496.