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The Potential Anti-Obesity Effect of Heated Stingless Bee Honey in Mice Fed a High-Carbohydrate High-Fat Diet

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Abstract

Stingless bee honey (SBH) has been widely recognized for its unique bioactive compounds and potential health benefits, including antioxidant and anti-inflammatory properties. However, the effects of heat processing on SBH's bioactivity, particularly its potential anti-obesity effects, remain inadequately explored. This study aims to evaluate the potential anti-obesity effects of heated SBH in mice fed a high-carbohydrate, high-fat (HCHF) diet. This study utilized 30 male white mice with an average weight of 19.03 ± 1.44 g were randomly divided into six groups (n=5). The animals were divided as follows: Group A (Normal Control): Mice fed a standard laboratory diet with no HCHF diet or SBH supplementation. Group B (HCHF Control): Mice fed an HCHF diet without SBH treatment. Group C (HCHF + Unheated SBH): Mice fed an HCHF diet and supplemented with unheated SBH. Group D (HCHF + Heated SBH at 50°C): Mice fed an HCHF diet and supplemented with SBH heated at 50°C. Group E (HCHF + Heated SBH at 75°C): Mice fed an HCHF diet and supplemented with SBH heated at 75°C. Group F (HCHF + Heated SBH at 90°C): Mice fed an HCHF diet and supplemented with SBH heated at 90°C. Mice were administered honey once daily orally for eight weeks. At the end of treatment, body weight, white adipose tissue accumulation, adipose tissue morphology and histology, blood glucose levels, total cholesterol, triglycerides, and HDL-cholesterol were measured. The results showed that mice fed HCHF without SBH administration had abnormal adipose tissue morphology and histology, while administration of heated SBH, especially at a temperature of 50°C, significantly improved the morphological and histological structure of adipose tissue under normal conditions. Administration of heated SBH also reduced adipose tissue weight, decreased fasting blood glucose levels, and increased lipid profiles. Thus, SBH, particularly when heated at 50°C, as a natural therapeutic agent for managing obesity.

Keywords: Adipose tissue, Anti-obesity, Blood glucose, Heating stingless bee honey, Lipid profile, Weight gain.

1. Introduction

Honey, produced by honey bees, is a natural and healthy product with significant health value. There are around 20,000 bee species, classified into numerous subfamilies. The genus *Apis*, subfamily Apinae, is the most well-known bee, producing huge quantities of honey whose identity and quality have been standardized [1, 2]. Another form of honey bee is the

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stingless honey bee, which belongs to the Meliponine subfamily and has around 500 species dispersed over tropical and subtropical regions [3, 4].

Stingless bee honey (SBH) is characterized by a sour, less sweet, and less viscous texture [5]. This honey may also be used to treat a variety of pathologies due to antioxidant activity, which is attributed to phenolic and flavonoid contents, rather than Apis honey [5, 6, 7]. Stingless bee honey, specifically *Heterotrigona itama*, is commonly grown in Latin America, Malaysia, Thailand, and Indonesia [8]. However, commercialization is still limited because of the limited quantity of honey output (1-3.5 kg per colony), as opposed to Apis honey (20 kg per colony) [9]. Furthermore, nothing is known about this honey, and industrial output is negligible. However, the fundamental disadvantage is that there is still no defined criterion to ensure the identification and quality of this honey [10, 11]. Furthermore, stingless bee honey degrades faster than Apis honey due to its physicochemical features, which include high water content and acidity [12]. Thus, identifying proper storage processing, avoiding fermentation or crystallization, and maintaining humidity to prevent the growth of certain fungi are key factors for growing the honey production chain. Heat treatment is one solution to the problem of SBH damage because it has no effect on honey quality and increases antioxidant capacity when heated at 90-95°C for 15-60 seconds [13]. The quality of stingless bee honey *Tetragonula laeviceps-pagdeni* was not significantly reduced when heated at 35 and 45°C for 60 minutes [14, 15]. Heating honey to the appropriate temperature can boost its phenolic content [8], which is one of the key components found in SBH. This phenolic molecule is anti-inflammatory, anti-obese, and reduces the development of insulin resistance [16, 17].

The impact of Apis honey supplementation on body weight and blood glucose levels in healthy rats, obese rats, and diabetic rats fed high sugar or high fat diets has been widely documented, with conflicting results [18, 19]. In general, research suggested that Apis honey supplementation has no significant effect on normal rats' body weight or blood glucose levels [20]. Supplementation with unheated SBH (which has different properties than Apis honey) had no significant effect on weight reduction or adipose cell size in obese rats [16, 20, 21]. Additionally, our early investigation showed that adding heated SBH to test animals in good health had no discernible impact on their rising body weight. In the event that heated SBH is consumed along with high-carbohydrate high-fat (HCHF) feed, there's a chance that the SBH could create unwanted side effects, including weight gain. This study was conducted to address a significant gap in the current understanding of how heated SBH influences metabolic outcomes when consumed alongside an HCHF diet. Although SBH has been recognized for its potential health benefits, limited research exists on how thermal processing of SBH affects its efficacy in modulating metabolic markers under diet-induced obesity conditions. The core issue under investigation is whether thermally processed SBH retains its protective or modulatory effects when combined with an obesogenic diet. The specific objectives of this study were: (I) to determine whether supplementation with heated SBH contributes to an increase in body weight and adipocyte size in mice fed with HCHF diet; (II) to evaluate whether it inhibits hyperglycemia; and (III) to assess its impact on lipid profile parameters, including total cholesterol, triglycerides, and high-density lipoprotein cholesterol (HDL-cholesterol) levels. To achieve these aims, a controlled experimental design was employed using an animal model, with detailed analysis of physiological and biochemical parameters following dietary intervention.

2. Materials and Methods

2.1 Ethical approval

All animal care, usage, and experimentation followed the Helsinki Declaration guidelines and the Andalas University Research Committee's established standards for the care and usage of experimental animals (No. 650/UN.16.2/KEP-FK/2022).

2.2 Honey collection procedure

Stingless bee honey *H. itama* was directly acquired from beekeepers in Jorong Ujuang Guguak, Saruaso Utara Village, Tanjung Emas District, Tanah Datar Regency, West Sumatra, Indonesia. Dark and thick-colored honey was specifically selected for this study. Honey was extracted from the beehives using a suction pump connected to a battery and a honey collection container. The honey was placed in plastic bottles and stored at ambient temperature. The honey collection was conducted in July 2022 during the rainy season.

2.3 Heat treatment

Heat treatment was performed following the protocol described by Braghini *et al.*, [8]. Three separate samples of honey (100 grams each) were placed into individual glass tubes and immersed in a thermostatic water bath (MTWB-1, Labomiz, United States). Each sample was heated separately at temperatures of $50\pm 2^\circ\text{C}$, $75\pm 2^\circ\text{C}$, and $90\pm 2^\circ\text{C}$ for 30 minutes. After heating, the honey samples were allowed to cool to room temperature and then stored in a refrigerator ($4 \pm 2^\circ\text{C}$) until further analysis.

2.4 Total phenolic content (TPC) measurement

The TPC was assessed using the Folin-Ciocalteu technique, as described by Ahmed and Khiati [22]. A 30 μL aliquot each of unheated and heated SBH solution (0.1 g/mL) was mixed with 2.37 mL of distilled water and 150 μL of 0.2 N Folin-Ciocalteu phenol reagent (Merck, Germany). A vortex was used to fully mix the solution, followed by incubating at the ambient temperature for two minutes. A 450 microliter of sodium carbonate solution (0.2 g/ml) (Merck, Germany) was added to the reaction mixture and kept for 2 hours at the ambient temperature. The absorption value was taken at 765 nm with a spectrophotometer (UV-VIS spectrophotometer Optima SP 300, Japan). The TPC was calculated by contrasting it to a standard curve created using gallic acid (0-200 mg/L) (Merck, Germany). The average of three measurements was determined and reported as mg equivalent of gallic acid (mg GAE) per 100 g of honey.

2.5 Animal subjects and high-carbohydrate and high-fat (HCHF) diet

2.5.1 Animals

Male white mice (*Mus musculus*) weighing 18-20 g were obtained from the Veterinary Center Animal House, Baso, West Sumatra, Indonesia. Mice were acclimatized for 6 days before treatment. Mice were housed individually in cages and provided with commercial feed (Rat Bio, PT Citrafeed, Indonesia) and water ad libitum.

2.5.2 Preparation of high-carbohydrate and high-fat (HCHF) diet

The HCHF diet was prepared based on a modified method from Ramli *et al.*, [23], and Rafie *et al.*, [16]. The HCHF diet consisted of 43 g of standard livestock feed, 25 g of fructose, and 32 g of solid beef fat (ghee). The feed was mixed until homogeneous (≈ 30 minutes).

2.6 Experimental design

The effect of SBH was examined in the Animal Zoology Laboratory, Department of Biology, UIN Mahmud Yunus Batusangkar. Heat-treated honey was administered to mice concurrently with an HCHF diet over a period of eight weeks. The experimental animals were randomly assigned to six treatment groups (n = 5), as outlined below:

Group A: Normal mice + Standard feed.

Group B: Normal mice + HCHF

Group C: Normal mice + HCHF + 500 mg/kg.bw unheated SBH.

Group D: Normal mice + HCHF + 500 mg/kg.bw heated SBH at 50°C

Group E: Normal mice + HCHF + 500 mg/kg.bw heated SBH at 75°C

Group F: Normal mice + HCHF + 500 mg/kg.bw heated SBH at 90°C

Mice were administered SBH once daily orally. Feed, HCHF diet and water were replaced daily. At the end of the treatment, total cholesterol, triglycerides, and high-density lipoprotein cholesterol (HDL-cholesterol) were measured using a digital lipid meter (Lipid Pro, Infopia Co., Ltd, Korea) and blood glucose was measured using a glucometer (GlukoDr, AGM-400, Korea). Furthermore, the mice were anaesthetized with chloroform, and the white adipose tissue (WAT) of all mice was collected and placed in 10% formalin for a short time.

2.7 Body weight measurement

Mice were weighed on an animal-specific digital scale (UW/UX 321-62150) before and after treatment.

2.8 White adipose tissue (WAT) weight measurement

White adipose tissue (WAT) was collected from the epididymal region of the mice at the end of the treatment period. Mice were euthanized by inhalation of chloroform vapor in a closed chamber, ensuring deep anesthesia followed by respiratory arrest in accordance with ethical guidelines for animal handling. WAT were weights on analytical-balances (Kern-ABJ 220-4NM, Germany).

2.9 Histological examination of white adipose tissue

WAT was obtained from the epididymal region of euthanized mice following chloroform anesthesia. WAT was subsequently isolated and fixed with 10% formaldehyde as experimental specimens. Preserved white adipose tissue was processed and embedded in paraffin wax. The tissue sections were stained with hematoxylin and eosin (H&E) before being analyzed under a light microscope (Olympus DP 22, Tokyo, Japan). Adipose tissue preparation was carried out at the Veterinary Laboratory, Baso, West Sumatra, Indonesia.

2.10 Blood glucose measurement

Blood glucose was measured at the end of the treatment using a digital glucometer (GlukoDr, AGM-400, Korea). Mice were fasted for approximately 9 hours. Blood was collected by cutting the tip of the mouse's tail and dropped onto the glucometer strip to read the blood glucose level [24].

2.11 Biochemical profile of lipid

Total cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL-cholesterol), and low-density lipoprotein cholesterol (LDL-cholesterol) levels were measured using a digital lipid meter (Lipid Pro, Infopia Co., Ltd, Korea) at the end of the treatment. Whole blood was collected from the mouse's tail, dropped onto the strip, and the values displayed on the Lipid Pro were recorded.

2.12 Analytical statistics

Statistical analysis was conducted using SPSS version 21.0 (IBM Corp., Armonk, NY, USA). One-Way Analysis of Variance (ANOVA) was employed to assess significant differences among the treatment groups. Post-hoc comparisons were performed using Duncan's New Multiple Range Test (DNMRT) at a 95% confidence level to identify which groups differed significantly. A p-value of less than 0.05 was considered statistically significant. Data are presented as mean \pm standard error of the mean (SEM).

3. Results

3.1 Total phenolic content (TPC)

The total phenolic content (TPC) of *Heterotrigona itama* stingless bee honey (SBH) exhibited significant variation following different heat treatments (Table 1). Overall, heat treatment enhanced the TPC, although the extent of the increase varied with temperature. Unheated SBH showed the lowest TPC value at 4.884 ± 0.051 mg AGE/g, which was significantly lower than all heat-treated samples (denoted by the letter *c*). Heating at 50°C resulted in a notable increase in TPC to 6.017 ± 0.221 mg AGE/g (denoted by *b*), while the highest TPC was observed at 75°C, reaching 6.526 ± 0.108 mg AGE/g (denoted by *a*), indicating a statistically significant enhancement compared to the unheated sample. At 90°C, the TPC slightly declined to 6.215 ± 0.095 mg AGE/g, which was not significantly different from the 75°C treatment (denoted by *ab*). This finding suggests that excessively high temperatures may lead to partial degradation of phenolic compounds, although the TPC remained higher than in the unheated honey. The observed increase in phenolic content at moderate heating levels (50 -75°C) may be due to the breakdown of complex structures or the release of bound phenolic compounds, making them more extractable. Conversely, thermal degradation at higher temperatures (e.g., 90°C) may account for the slight reduction in TPC.

Table 1: TPC of SBH heated at different temperatures

Treatment	Total Phenolic (mg AGE/g)
Unheated SBH	4.884 ± 0.051 c
Heated SBH at 50 °C	6.017 ± 0.221 b
Heated SBH at 75 °C	6.526 ± 0.108 a
Heated SBH at 90 °C	6.215 ± 0.095 ab

Note: Values are expressed as mean \pm SEM (n = 3). Different superscript letters indicate significant differences ($p < 0.05$) between groups.

3.2 The morphology and histology of white adipose tissue (WAT)

Figure 1 shows the effect of SBH supplementation heated at different temperatures on the morphology and histology of epididymal white adipose tissue (WAT) of mice fed HCHF after 8 weeks of treatment compared to control mice.

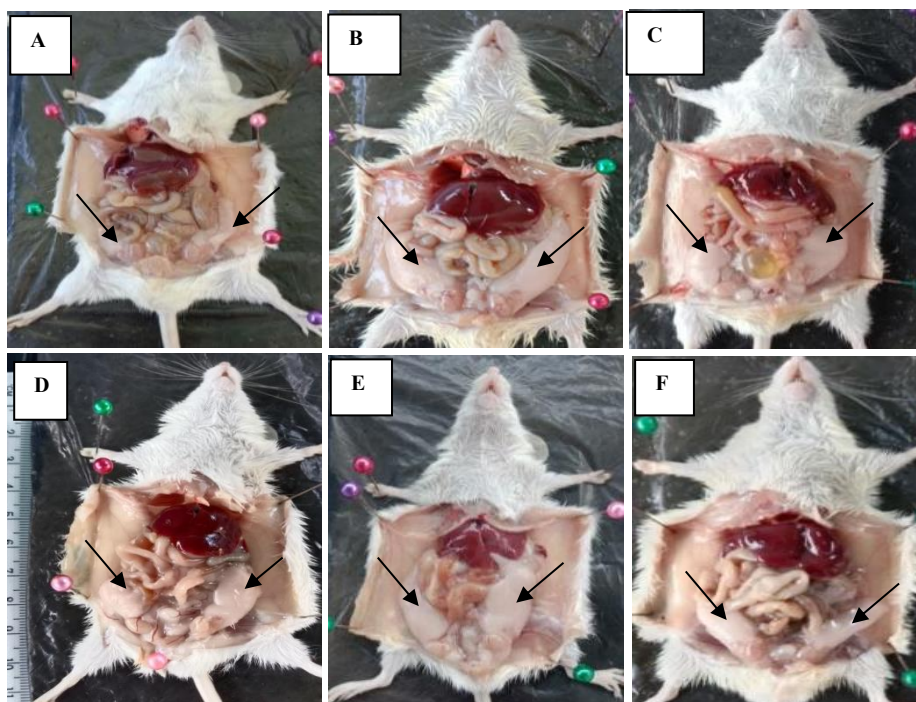


Figure 1: Morphology of epididymal white adipose tissue (WAT) in normal mice fed a HCHF diet and supplemented with heated SBH at different temperatures. A: Normal mice + Standard feed; B: Normal mice + HCHF; C: Normal mice + HCHF + 500 mg/kg.bw unheated SBH; D: Normal mice + HCHF + 500 mg/kg.bw heated SBH at 50°C; E: Normal mice + HCHF + 500 mg/kg.bw heated SBH at 75°C; F: Normal mice + HCHF + 500 mg/kg.bw heated SBH at 90°C. The images were captured using a VIVO 2019 smartphone camera (48 MP resolution). The displayed images represent all the studied animals. Black arrows indicate the location of epididymal WAT.

Comparison of WAT morphology revealed a clear distinction between mice fed a normal diet (Figure 1A) and those subjected to an HCHF diet (Figure 1B). In mice receiving the normal diet, WAT appeared small and loosely arranged, indicating a healthy adipose structure. In contrast, the HCHF-fed group exhibited noticeably enlarged and denser WAT, suggesting excessive lipid accumulation as a result of the high-energy diet. When unheated SBH was administered in combination with the HCHF diet (Figure 1C), the WAT morphology showed improvement compared to the HCHF group. Adipocytes appeared smaller, and the amount of accumulated adipose tissue was reduced, indicating that unheated SBH exerted a protective effect against adiposity enlargement in HCHF-fed mice. This beneficial effect was also observed in the group treated with SBH heated at 50°C (Figure 1D). However, no such improvement was seen in the groups receiving SBH heated at 75°C and 90°C (Figures 1E and F). The WAT in these groups appeared larger and denser, similar to the HCHF group. These findings suggest that heat treatment degrades or eliminates bioactive compounds in SBH that are responsible for inhibiting lipid accumulation, thereby reducing its effectiveness in preventing adipose tissue expansion.

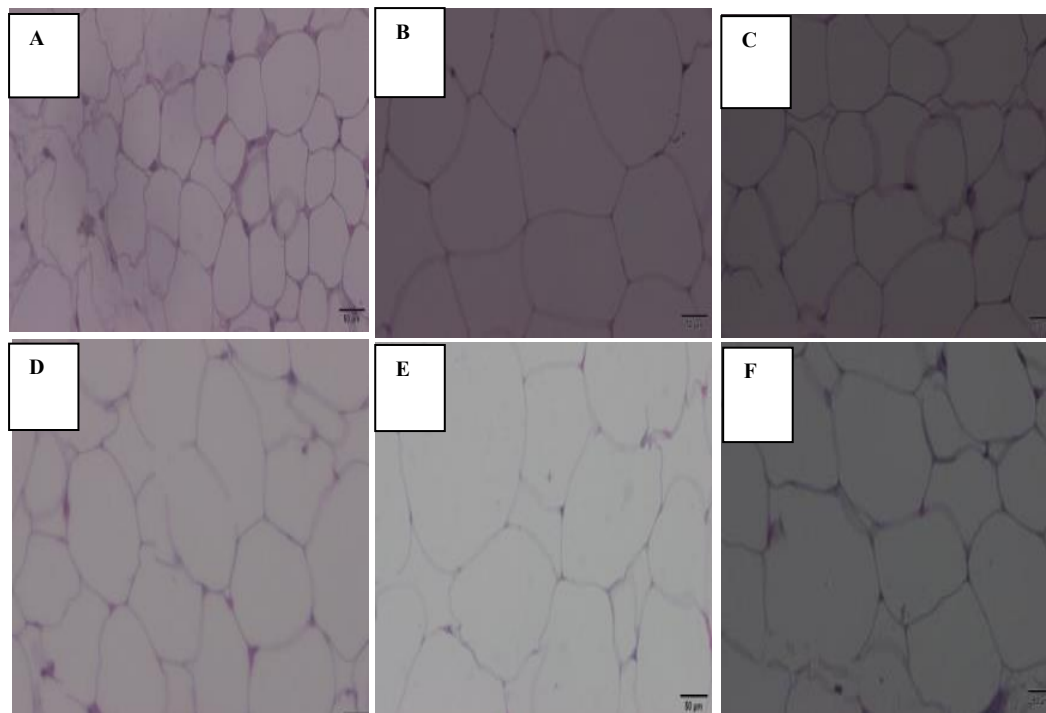


Figure 2: Histology of epididymal white adipose tissue (WAT) in normal mice fed a HCHF diet and supplemented with heated SBH at different temperatures. A: Normal mice + Standard feed; B: Normal mice + HCHF; C: Normal mice + HCHF + 500 mg/kg.bw unheated SBH; D: Normal mice + HCHF + 500 mg/kg.bw heated SBH at 50°C; E: Normal mice + HCHF + 500 mg/kg.bw heated SBH at 75°C; F: Normal mice + HCHF + 500 mg/kg.bw heated SBH at 90°C. The tissue was stained with H & E and captured at 40X. The displayed images represent all the studied animals

The histological evaluation of epididymal adipose tissue demonstrated clear variations across different treatment groups. In rats maintained on a standard diet (Figure 2A), the adipose cells were small, dispersed loosely, and exhibited size variability, indicative of normal, healthy adipose architecture. Conversely, in the group subjected to an HCHF diet (Figure 2B), the adipocytes were notably enlarged and showed a uniform shape, consistent with lipid overload due to excessive caloric intake. Unheated SBH alongside the HCHF diet (Figure 2C) resulted in significant structural improvements, where adipocytes were smaller, irregular in size, and less densely compacted. This suggests a mitigating effect of unheated SBH against adipocyte hypertrophy induced by the obesogenic diet. Similar benefits, though to a lesser extent, were observed in the group administered SBH that had been heated to 50°C (Figure 2D), indicating that partial thermal stability of bioactive compounds may be retained at this temperature. In contrast, SBH heated to 75°C and 90°C (Figures 2E and 2F) failed to produce similar improvements. In these groups, adipocytes appeared large and uniform, closely resembling those in the HCHF-only group, implying that elevated temperatures may deactivate critical bioactive components in SBH. Overall, these findings highlight that the protective effect of SBH on adipocyte morphology is significantly influenced by the degree of heat exposure during processing.

3.3. Body weight and white adipose tissue (WAT) weight

A comparison between the normal diet group (Group A) and the HCHF diet group (Group B) revealed significant alterations in both body and WAT weights. Although the body weight of mice in the HCHF group (34.50 ± 0.96 g) was slightly lower than that of the normal diet

group (36.75 ± 1.97 g), the WAT weight markedly increased from 0.63 ± 0.17 g to 1.36 ± 0.12 g. This indicates that the HCHF diet promotes visceral fat accumulation despite the absence of a corresponding gain in total body weight. When comparing the HCHF group supplemented with unheated honey (Group C) to those receiving heated honey (Groups D, E, and F), a notable difference in physiological response was observed. In Group C, WAT weight decreased to 0.86 ± 0.71 g, suggesting a protective effect of unheated honey against HCHF-induced fat accumulation. Although body weight slightly decreased to 33.00 ± 2.04 g, it remained statistically comparable to Group B. Conversely, in groups administered heated honey, the protective effect was less apparent. While body weights continued to decline (32.00 ± 1.22 g in Group D; 31.00 ± 1.82 g in Group E; and 31.00 ± 2.58 g in Group F), WAT mass remained relatively high, particularly in the 75°C (1.24 ± 0.04 g) and 90°C (1.07 ± 0.05 g) treatment groups. Comparative analysis among the heated honey groups indicated that honey heated to 50°C (Group D) retained some protective efficacy, as evidenced by the relatively low WAT weight (0.87 ± 0.08 g), which was comparable to that in Group C. However, this effect was diminished or lost entirely at higher heating temperatures (75°C and 90°C), where WAT weight increased significantly, resembling the values observed in the HCHF-only group.

Collectively, these findings indicate that SBH possesses notable anti-adipogenic properties in a model of diet-induced obesity. The protective effects are most evident when SBH is administered in its unheated form or after moderate thermal treatment (up to 50°C). Heating above 50°C significantly diminishes these effects, likely due to the degradation of key bioactive compounds. Therefore, thermal processing emerges as a critical factor influencing the therapeutic efficacy of SBH in mitigating visceral fat accumulation and obesity-related metabolic alterations.

Table 2: Body weight and WAT weight of mice fed HCHF diet and supplemented with heated SBH at different temperatures

Groups	Body Weight (g)	WAT Weight (g)
A (Normal mice + Standard feed)	36.75 ± 1.97^a	0.63 ± 0.17^c
B (Normal mice + HCHF)	34.50 ± 0.96^{ab}	1.36 ± 0.12^a
C (Normal mice + HCHF + 500 mg/kg.bw unheated SBH)	33.00 ± 2.04^{ab}	0.86 ± 0.71^{bc}
D (Normal mice + HCHF + 500 mg/kg.bw heated SBH at 50°C)	32.00 ± 1.22^b	0.87 ± 0.08^{bc}
E (Normal mice + HCHF + 500 mg/kg.bw heated SBH at 75°C)	31.00 ± 1.82^b	1.24 ± 0.04^a
F (Normal mice + HCHF + 500 mg/kg.bw heated SBH at 90°C)	31.00 ± 2.58^b	1.07 ± 0.05^{ab}

Note: Values are expressed as mean \pm SEM (n = 5). Different superscript letters indicate significant differences ($p < 0.05$) between groups

3.4. Fasting blood glucose levels

The effect of heated SBH supplementation on fasting blood glucose (FBG) levels in all experimental mice groups after eight weeks of treatment. The result showed that heated SBH supplementation has a significant effect ($p < 0.05$) on FBG when compared to HCHF mice (Group B), indicating that both dietary composition and honey supplementation influenced glucose metabolism (Figure 2). Mice fed a standard diet (Figure 2, Group A) had the lowest

fasting blood glucose levels (~75 mg/dL), reflecting a normal and healthy metabolic state. In contrast, mice given an HCHF diet (Figure 2, Group B) exhibited significantly elevated glucose levels (~125 mg/dL), consistent with diet-induced hyperglycemia. Supplementation with unheated SBH in HCHF-fed mice (Figure 2, Group C) reduced blood glucose levels to approximately 110 mg/dL, suggesting a protective effect of honey in regulating glycemia. Interestingly, SBH heated to 50°C (Figure 2, Group D) produced an even greater reduction in glucose levels (~95 mg/dL), indicating that the key bioactive compounds responsible for its antihyperglycemic effects remained active at this temperature. However, this beneficial effect was diminished when SBH was heated to higher temperatures. Mice in Groups E and F, which received SBH heated to 75°C and 90°C respectively, showed higher glucose levels (~100–105 mg/dL) than Group D. Although these levels were still lower than those in the HCHF-only group, the results suggest that higher temperatures may partially degrade the honey's bioactive components. These findings highlight the antihyperglycemic potential of SBH in managing glucose levels in HCHF-induced metabolic disturbances. Importantly, its effectiveness is temperature-dependent, with optimal benefits observed at 50°C.

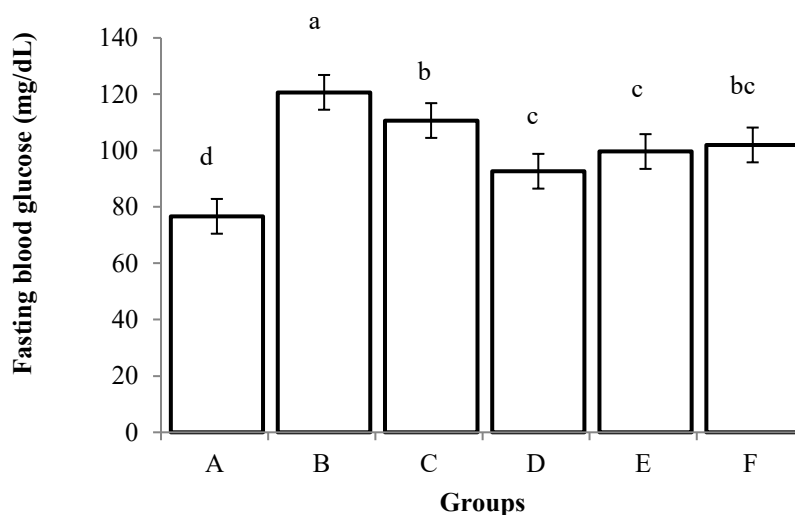


Figure 3: Fasting blood glucose of mice fed HCHF diet and supplemented with heated SBH at different temperatures. A: Normal mice + Standard feed; B: Normal mice + HCHF; C: Normal mice + HCHF + 500 mg/kg.bw unheated SBH; D: Normal mice + HCHF + 500 mg/kg.bw heated SBH at 50°C; E: Normal mice + HCHF + 500 mg/kg.bw heated SBH at 75°C; F: Normal mice + HCHF + 500 mg/kg.bw heated SBH at 90°C. Values are expressed as mean \pm SEM (n = 5). Different superscript letters indicate significant differences ($p < 0.05$) between groups

3.5. Lipid profile

The effect of heated SBH supplementation on the blood lipid profile of experimental mice after 8 weeks is presented in Table 3. Mice fed with the HCHF diet (Group B) exhibited clear signs of dyslipidemia, with a significant increase in total cholesterol (131.33 ± 1.20 mg/dL) and triglyceride levels (142.67 ± 13.92 mg/dL), accompanied by a significant decrease in HDL-cholesterol (44.33 ± 1.20 mg/dL), compared to the control group (Group A) ($p < 0.05$). Supplementation with unheated SBH (Group C) significantly improved the lipid profile, reducing total cholesterol to 106.00 ± 3.46 mg/dL and increasing HDL-cholesterol to 60.00 ± 1.15 mg/dL ($p < 0.05$). Triglyceride levels also decreased to 80.67 ± 7.22 mg/dL, although the reduction was less pronounced than in the heated SBH groups. The most substantial

improvement in lipid parameters was observed in Group D, which received SBH heated at 50°C. This group showed the lowest triglyceride level (67.00 ± 4.93 mg/dL) and a reduced total cholesterol level (106.67 ± 3.51 mg/dL), both significantly lower than in the HCHF group ($p < 0.05$). HDL-cholesterol was markedly increased to 66.33 ± 2.73 mg/dL, indicating strong lipid-protective effects. In Group E (SBH heated at 75°C), a slight increase in total cholesterol (115.67 ± 2.03 mg/dL) and triglycerides (82.00 ± 3.21 mg/dL) was noted compared to the 50°C group; however, HDL-cholesterol remained high (65.33 ± 1.45 mg/dL). Interestingly, Group F, which received SBH heated at 90°C, exhibited the highest HDL-cholesterol level among all groups (69.33 ± 4.67 mg/dL; $p < 0.05$). However, this group also showed a moderate increase in total cholesterol (111.33 ± 5.69 mg/dL) and triglyceride levels (92.00 ± 4.36 mg/dL) compared to groups treated at lower heating temperatures. These findings indicate that SBH supplementation, particularly when heated at 50°C, significantly improves the lipid profile in mice subjected to an HCHF diet. This treatment effectively reduces total cholesterol and triglyceride levels while increasing HDL-cholesterol, with the most optimal effects observed at the moderate heating temperature.

Table 3: The lipid profile of mice fed HCHF diet and supplemented with heated SBH at different temperatures.

Groups	Lipid Profile		
	Total Cholesterol (mg/dL)	Trygliceride (mg/dL)	HDL-Cholesterol (mg/dL)
A (Normal mice + Standard feed)	95.00 ± 3.21 ^c	58.33 ± 1.45 ^d	55.00 ± 2.65 ^b
B (Normal mice + HCHF)	131.33 ± 1.20 ^a	142.67 ± 13.92 ^a	44.33 ± 1.20 ^d
C (Normal mice + HCHF + 500 mg/kg.bw unheated SBH)	106.00 ± 3.46 ^b	80.67 ± 7.22 ^{bed}	60.00 ± 1.15 ^{bc}
D (Normal mice + HCHF + 500 mg/kg.bw heated SBH at 50°C)	106.67 ± 3.51 ^c	67.00 ± 4.93 ^{cd}	66.33 ± 2.73 ^{ab}
E (Normal mice + HCHF + 500 mg/kg.bw heated SBH at 75°C)	115.67 ± 2.03 ^b	82.00 ± 3.21 ^{bc}	65.33 ± 1.45 ^{ab}
F (Normal mice + HCHF + 500 mg/kg.bw heated SBH at 90°C)	111.33 ± 5.69 ^a	92.00 ± 4.36 ^b	69.33 ± 4.67 ^a

Note: Values are expressed as mean \pm SEM (n = 3). Different superscript letters indicate significant differences ($p < 0.05$) between groups.

4. Discussion

The findings of this study clearly show that heat treatment significantly influences the total phenolic content (TPC) of *Heterotrigona itama* SBH, with the most beneficial effects observed at moderate temperatures. Heating at 50°C led to a marked increase in TPC, which peaked at 75°C. This suggests that moderate thermal processing may facilitate the release of bound phenolic compounds or cause structural changes in the honey matrix that enhance phenolic extractability. These results are consistent with previous research indicating that controlled heating can break down complex structures, allowing more phenolic compounds to be released [25]. Interestingly, a slight decrease in TPC was observed at 90°C, even though the value remained higher than in the unheated sample. This indicates that at higher temperatures, thermal degradation may begin to occur. Phenolic compounds are known to be sensitive to heat, and excessive temperatures may lead to oxidative degradation or

polymerization, reducing their biological availability [26]. Therefore, optimizing the heating temperature is essential to preserve and enhance the functional properties of SBH. Phenolics are one of the main bioactive compounds in SBH. They play a vital role as antioxidants and are known to help prevent metabolic disorders such as obesity and diabetes mellitus by reducing body weight, fat mass, and adipocyte size, as well as improving insulin sensitivity [16]. Heating SBH at the appropriate temperature increases its phenolic content [13], which may strengthen its therapeutic effects. Phenolics help reduce oxidative stress caused by HCHF diets. Since oxidative stress contributes to insulin resistance and inflammation, two key factors in the development of obesity and diabetes, phenolics may support better metabolic health by reducing these harmful processes [27].

The administration of SBH heated at 50°C in combination with HCHF diet led to marked improvements in both the morphology and histology of WAT. Histological analysis revealed smaller adipocyte size, irregular cellular architecture, and reduced adipocyte density, accompanied by decreased lipid accumulation. These morphological alterations suggest that heated SBH may exert a modulatory effect on adipose tissue expansion under obesogenic dietary conditions. The observed protective effects could be attributed to the retention of key bioactive compounds, such as phenolics and flavonoids, which are known to exhibit antioxidant and anti-inflammatory properties even after mild thermal processing [13, 27]. The reduction in adipocyte hypertrophy is particularly significant, as enlarged adipocytes are metabolically dysfunctional and contribute to systemic insulin resistance and chronic low-grade inflammation, key features of metabolic syndrome [28]. By mitigating adipocyte expansion and improving tissue architecture, SBH likely plays a role in preserving adipose tissue homeostasis. Similar findings have been reported in other studies investigating natural products rich in polyphenols, which have demonstrated the ability to attenuate adipose tissue remodeling and lipid accumulation in diet-induced obese models [16]. Therefore, the results suggest that SBH, even when subjected to moderate heating at 50°C, retains functional bioactivity that contributes to its anti-adipogenic effects, supporting its potential use as a functional dietary adjunct in the management of obesity and related metabolic disorders.

Heating SBH up to 50°C preserves its protective effects against diet-induced adiposity, characterized by lower body weight and adipose tissue weight compared to without honey administration. At this temperature, key bioactive compounds such as polyphenols and flavonoids likely remain stable, allowing SBH to retain its ability to modulate lipid metabolism and inhibit adipogenesis. Thus, 50°C appears to be a critical threshold for maintaining the functional integrity of honey's thermosensitive constituents, supporting its potential role as a natural dietary adjunct in the prevention of obesity. This observation aligns with prior studies indicating that certain phenolic compounds, flavonoids, and enzymatic antioxidants in honey remain relatively stable at moderate heating ($\leq 50^{\circ}\text{C}$), thereby retaining their biological function in modulating lipid metabolism and adipogenesis [25]. The potential mechanisms may involve the inhibition of lipogenesis-related gene expression and the enhancement of lipid oxidation pathways, as previously reported in studies involving natural polyphenol-rich substances [28]. These findings support the hypothesis that honey, when processed at controlled temperatures, may exert protective effects against diet-induced adiposity by preserving its thermosensitive constituents, such as glucose oxidase, and polyphenolic antioxidants [16].

The present study demonstrates that SBH, when heated at a moderate temperature of 50°C, significantly lowers fasting blood glucose levels in mice fed a HCHF diet. This suggests that SBH retains key bioactive compounds, particularly phenolics and flavonoids, at this temperature, which likely contribute to its hypoglycemic effects. These compounds are known for their antioxidant and anti-inflammatory activities, which help mitigate oxidative stress and chronic inflammation, both of which are central to insulin resistance and glucose dysregulation. Prior studies have shown that natural honey can improve glycemic control through similar mechanisms, including modulation of insulin signaling and suppression of pro-inflammatory cytokines [29]. Anyukado *et al.*, reported that diabetic rats receiving a honey-based diet exhibited significantly improved plasma glucose regulation compared to those fed a standard diet [30]. Similarly, Erejuwa *et al.*, found that Tualang honey supplementation was able to maintain normal insulin concentrations in diabetic models, indicating honey's potential in preserving insulin functionality. Importantly, the glucose-lowering effect of SBH observed in this study may extend its relevance to obesity-related metabolic dysfunction [31]. Chronic hyperglycemia contributes to increased de novo lipogenesis, adipocyte hypertrophy, and ectopic fat deposition, all of which exacerbate insulin resistance and promote weight gain [32]. By reducing circulating glucose levels, SBH may indirectly attenuate these obesogenic mechanisms and contribute to improved metabolic homeostasis [30]. Furthermore, its antioxidant and anti-inflammatory properties may protect adipose tissue from oxidative insults and inflammatory signaling, thereby enhancing insulin sensitivity. These findings suggest that thermally optimized SBH may serve as a functional dietary agent not only for glycemic control but also as a complementary strategy in obesity management.

In addition to its hypoglycemic effects, SBH supplementation, particularly when heated at 50°C, was found to significantly improve the lipid profile in mice subjected to a HCHF diet. This beneficial outcome is likely attributed to the preservation of key bioactive constituents, such as phenolic compounds, flavonoids and enzymes, which are known to exhibit lipid-lowering effects. These compounds function by modulating lipid metabolism pathways, including inhibition of hepatic lipogenesis, enhancement of fatty acid β -oxidation, and improvement in bile acid excretion, ultimately contributing to reductions in serum total cholesterol, low-density lipoprotein cholesterol (LDL-C), and triglyceride (TG) levels, while increasing high-density lipoprotein cholesterol (HDL-C) concentrations [17, 28]. Phenolic compounds and other flavonoids present in SBH also exert antioxidative effects by reducing lipid peroxidation, which plays a pivotal role in the pathogenesis of dyslipidemia in obesity and metabolic syndrome. The antioxidant activity of SBH may prevent the oxidation of LDL particles, thereby reducing atherogenic risk and improving cardiovascular health [33]. Furthermore, the anti-inflammatory properties of SBH may contribute to a healthier lipid profile by downregulating pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF- α) and interleukin-6 (IL-6), which are known to impair lipid handling in hepatocytes and adipose tissue [34]. Previous studies have demonstrated that honey supplementation improves lipid parameters in both diabetic and hyperlipidemic models, supporting its potential as a natural therapeutic agent [35].

Based on the cumulative findings, SBH heated at 50°C demonstrates potential as a natural anti-obesity agent and may serve as an alternative or complementary strategy to conventional obesity therapies. This includes notable improvements in favorable modulation of adipose tissue morphology, enhancements in glycemic control, and lipid profile. These results are consistent with earlier reports highlighting the stability of certain bioactive constituents in honey under controlled heating conditions, which may lead to enhanced phenolic availability

and synergistic biological activity [13, 17, 28]. The therapeutic effects of SBH are likely mediated by antioxidant, anti-inflammatory, and metabolic gene regulation pathways.

Despite the promising outcomes observed, this study is not without limitations, and further research is warranted to fully elucidate the molecular mechanisms underlying the beneficial effects of thermally treated SBH. Detailed investigation into gene expression profiles, enzymatic activity, and inflammatory signaling pathways would help clarify the specific bioactive pathways modulated by SBH. In addition, long-term studies involving human subjects are essential to verify the translatability of these findings from animal models to clinical settings. Moreover, it remains crucial to standardize SBH composition and identify potential thresholds of thermal exposure beyond which its therapeutic properties might be compromised. Nevertheless, based on the current findings, we propose that moderately heated SBH holds considerable potential as a natural, cost-effective adjunct in the prevention and management of obesity and related metabolic disorders, especially in populations seeking alternative and complementary nutritional therapies.

5. Conclusion

This study highlights the potential of SBH, particularly when heated at 50°C, as a natural therapeutic agent for managing obesity and related metabolic disorders. Moderate heating at this temperature was shown to enhance the honey's phenolic content, preserving key bioactive compounds such as flavonoids and enzymes that contribute to improved antioxidant and anti-inflammatory activity. These effects translated into significant physiological benefits, including reduced adiposity, improved adipose tissue architecture, lower fasting blood glucose, and a more favorable lipid profile in mice fed a HCHF diet. The findings suggest that 50°C represents an optimal threshold for maintaining the functional integrity of SBH's thermosensitive components. However, further studies are needed to confirm the underlying molecular mechanisms and validate the clinical relevance of these effects in humans. Overall, thermally optimized SBH may serve as an effective, accessible, and complementary dietary intervention for metabolic health management.

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Conflict of interest statement

The authors declare that they have no conflicts of interest.

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