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Comparative analysis of anti-obesity effects of green, fermented, and γ-aminobutyric acid teas in a high-fat diet-induced mouse model

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Abstract

Obesity, a prevalent disease associated with numerous chronic conditions, including hyperlipidemia, hyperglycemia, diabetes, and metabolic syndrome, remains a major global health challenge. This study investigated the potential of green tea (GT), fermented tea (FT), and γ-aminobutyric acid (GABA) tea (GBT), which are rich in phytonutrients and polyphenols, for the management of obesity. Using a high-fat diet-induced obese mouse model (C57BL/6N), we explored the effect of these teas on various obesity-related parameters. The mice were categorized into five groups: normal diet with water, high-fat diet with water, and high-fat diet supplemented with GT, FT, or GBT. Over 13 weeks, we monitored body weight, perirenal and liver fat, adipocyte lipid accumulation, and key metabolic indicators, such as serum cholesterol, leptin, insulin, and fasting blood glucose. These teas contain beneficial phytochemicals such as GABA, theanine, and caffeine, and have demonstrated an enhanced antioxidant capacity, which increases the scavenging of free radicals and may reduce oxidative stress. The animal study indicated a decrease in feeding efficiency and significant reductions in body weight liver fat, epididymal fat, and perirenal fat, as well as in adipocyte lipid accumulation. Additionally, notable improvements were observed in metabolic health indicators, including reductions in serum cholesterol, leptin, insulin, and fasting blood glucose levels. Our findings revealed that GT, FT, or GBT significantly counteracted the negative effects of a high-fat diet, suggesting their potential in combating obesity and related metabolic disorders.

Keywords Anti-obesity effects, Green tea, Fermented tea, GABA tea, High-fat diet-induced obesity

Introduction

Obesity, defined as excessive fat accumulation, is a critical health concern worldwide. Due to an imbalance in energy intake and alterations in gut microbiota compounded by genetic and environmental factors, the prevalence of obesity has surged globally [1]. Since 1980, the number of individuals with obesity has doubled, affecting approximately one-third of the global population [2]. This condition is linked to various chronic diseases, including hyperlipidemia, hyperglycemia, diabetes, and metabolic syndromes [3]. Despite the numerous drugs available for the treatment of obesity, they often have multiple side effects [4].

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In existing studies on obesity, a consolidated set of indicators has been pivotal in delineating the complex interplay of metabolic and hormonal factors inherent in this condition [5]. Adipocyte lipid accumulation is recognized for its role in signifying energy imbalance and fat storage, while serum cholesterol levels are examined due to their correlation with obesity-related cardiovascular risks [6]. Leptin, a hormone signaling satiety and energy balance, alongside insulin, which is central to glucose homeostasis, is critically assessed to understand the dysregulations typical of obesity [7]. Furthermore, fasting blood glucose levels are monitored as they reflect the body's ability to regulate sugar levels, providing insights into the metabolic disturbances frequently accompanying obesity [8]. Collectively, these indicators form the cornerstone of comprehensive obesity research, enabling a multifaceted understanding of its pathophysiology and guiding the development of targeted interventions.

Tea, beyond its basic nutritional value, is acclaimed for its beneficial health impacts. As a widely consumed beverage globally, it plays a significant role in promoting healthy aging and preventing chronic diseases [9–11]. Green tea, scientifically known as Camellia sinensis, is rich in polyphenols, such as epigallocatechin-3-gallate (EGCG). It is prepared in an unfermented form, involving the drying and steaming of fresh leaves to preserve the polyphenol content [12]. The role of post-fermented tea, derived through microbial fermentation, in reducing the risk of hyperlipidemia and atherosclerosis has been increasingly acknowledged [13]. γ-aminobutyric acid (GABA), a naturally occurring amino acid in tea, augments polyphenol concentrations and antioxidant capabilities, especially under heat stress [14]. These teas, laden with various bioactive compounds, are often termed "functional tea" due to their wide-ranging health benefits.

Tea polyphenols, known as catechins, are flavonoids with a distinctive α-phenyl-benzopyran structure [15]. These polyphenols, including EGCG, epicate-chin-3-gallate (ECG), epigallocatechin (EGC), and epicatechin (EC), are categorized as ester and non-ester catechins [16]. The antioxidant properties of catechins depend on their molecular structure, particularly their hydroxyl group arrangement [17]. Their unique 4-oxo, 3-hydroxy C ring structure enhances their resistance to oxidation [18, 19].

In this study, we evaluated the anti-obesity effects of GT, FT, and GBT in a high-fat diet-induced obesity model using C57BL/6 mice. We examined the effect of these tea varieties on weight management and metabolic health indicators to provide insights into their potential as therapeutic agents for obesity.

Materials and methods

Experimental materials

Tea leaves harvested in May 2020 from Hadong, Korea, were processed to produce various types of tea. The preparation of GT and FT followed the methods detailed in the previous study [20]. Specifically, GT processing began with pan-firing fresh leaves at 250–300 °C for 10–15 min to halt enzymatic oxidation. This was followed by rolling the leaves, a 10 min cooling period, and two drying stages at 120–150 °C, then at 70–100 °C. In contrast, the FT underwent a different process: the leaves were withered in the sun for 1–2 h, rolled for 15 min, and then left to oxidize naturally in sunlight. This oxidation step was repeated three times before the leaves were finally sun-dried.

GBT was produced by subjecting the freshly harvested leaves to an anaerobic environment at room temperature for 24 h to increase GABA content, then airing them to dissipate any off-flavors for two hours, and subsequently processing them using the same methods as for GT.

For the extraction, 20 g of each tea variant was steeped in 2 L of distilled water. The mixture was then agitated at 100 rpm and 25 °C for 30 min using a shaking incubator (IS-971R, JS Research Inc., Gongju-si, Korea). After incubation, the extracts were filtered with Whatman No. 2 filters and stored at 4 °C for subsequent analysis and experimentation.

Amino acid analysis

To analyze the amino acid content, 0.3 g of the powdered sample was combined with 10 mL of distilled water and subjected to extraction at 37 °C for 24 h. Following the extraction, the mixture was filtered, and the filtrate was then concentrated using a rotary evaporator (Eyela N-1100, Tokyo Rikakikai Co., Ltd., Tokyo, Japan). The resulting concentrate underwent treatment with 1 mL of 5-sulfosalicylic acid dihydrate and was allowed to settle at 4 °C in a refrigerator for another 24 h. After this incubation period, the sample was centrifuged at 4000 rpm for 15 min. The supernatant obtained was again concentrated under vacuum at temperatures not exceeding 40 °C. This concentrated sample was then reconstituted in 5 mL of 0.2 M Lithium citrate buffer (pH 2.2) and passed through a 0.2 μm membrane filter for final clarification. A 100 μL aliquot of the filtered sample was subjected to analysis on an amino acid analyzer (Sykam S-433 D, Sykam GmbH, Eresing, Germany) that features an S7130 amino acid reagent organizer, S5200 sample injector, and S2100 solvent delivery system for precise amino acid quantification. Chromatographic separation was achieved using a cation exchange column LCA K06/NA (4.6 mm × 250 mm). The chromatography was performed with a mobile phase flow rate set at 0.45 mL/min and a ninhydrin flow rate at

0.4 mL/min. The quantification of individual amino acids was conducted based on the comparison with a standard amino acid solution, utilizing the analyzer's integrated software for accurate determination of each amino acid's concentration.

Quantitative analysis of caffeine and catechins in tea extracts

Standard compounds for catechin and caffeine analyses were procured from Sigma-Aldrich (St. Louis, MO, USA). All solvents used for the HPLC analysis were of analytical grade. Tea samples were first ground into a fine powder. Precisely 0.5 g of this powdered sample was weighed and mixed with 50 mL of 50% (v/v) ethanol. The mixture underwent ultrasonic extraction (Ultrasonic, Seong Dong Co., Seoul, Korea) at 37 °C for 1 h and 30 min to effectively extract catechins and caffeine. Following extraction, the solution was filtered through Whatman No. 2 filter paper (Maidstone, UK) and concentrated via a rotary evaporator (Eyela N-1100, Tokyo Rikakikai Co., Ltd., Tokyo, Japan). This extraction and concentration process was repeated three times to ensure maximum yield. The final concentrate was redissolved in 100% methanol, and the solution was then filtered through a 0.45 μm membrane filter (Advantec, Toyo Roshi Kaisha Ltd, Tokyo, Japan) for HPLC analysis. The quantitative determination of catechins and caffeine was performed using an HPLC system (Dionex Ultimate 3000, Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA). Chromatographic separation was achieved on a Kinetex C18 column (5 μm, 250 mm × 4.6 mm, Phenomenex, Torrance, California, USA) equipped with a Photodiode Array Detector (PDA). The column temperature was maintained at 40 °C throughout the analysis. The mobile phase consisted of 0.1% phosphoric acid in water (Solvent A) and acetonitrile (Solvent B). The gradient program started with 85% A (0–2 min), changed to 70% A (2–40 min), then to 0% A (40–42 min), and returned to 85% A (42–44 min), with a post-time of 3 min at 85% A to re-equilibrate the column (44–47 min). The flow rate was maintained at 1 mL/min, and the injection volume for each analysis was set at 10 μL. The content of catechins and caffeine in the tea samples was quantified by comparing their peak areas to those of the corresponding standards, with detection performed by the PDA detector across a suitable wavelength range.

Measurement of total polyphenol content

Total polyphenol content was determined utilizing a modified Folin-Ciocalteu method, as referenced in [21]. The functional teas of 2.0 g were subjected to extraction with 80 ml of 50% EtOH for one hour. Post extraction, the sample underwent concentration and freeze-drying processes. The freeze-dried material was then reconstituted to a concentration of 0.25 mg/mL, 0.5 mg/mL, and 1 mg/mL. To 0.5 mL of these solutions, 0.5 mL of Folin-Ciocalteu reagent (from Fluka, Buchs, Switzerland) was added, mixed, and incubated at ambient temperature for five minutes. After this period, 4.5 mL of 7% sodium carbonate solution was incorporated and allowed to stand for an additional five minutes. Subsequently, 2 mL of distilled water was added, and the mixture was incubated in darkness for 30 min. Upon completion, the supernatant was extracted, and its absorbance was recorded at 760 nm utilizing a microplate reader (Epoch2; BioTek Instruments, Inc., Winooski, VT, USA). The quantification was based on a gallic acid standard (Sigma-Aldrich Co.), with results expressed in mg of gallic acid equivalent (GAE) per g of dry sample.

Evaluation of antioxidant activity in tea extracts using DPPH and ABTS assays

Following the procedures utilized in the previous study [22], we assessed the antioxidant activities of GT, FT, and GBT using DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis[3-ethylbenzothiazoline-6-sulfonic acid]) assays, which measure the scavenging abilities of the extracts against stable free radicals. In both assays, the tea extracts were introduced to radical solutions, and the reduction in absorbance, which indicates the neutralization of radicals, was measured at 517 nm for DPPH and 734 nm for ABTS. The decrease in color intensity is proportional to the extracts' antioxidant capacities, enabling a comprehensive evaluation of their radical-scavenging potential in both hydrophilic and lipophilic environments.

Experimental animals

We procured 35 male C57BL/6N mice, aged 3 weeks, from KOATECH (Pyeongtaek City, Korea). Mice were housed in groups of seven in cages measuring 500 × 300 × 200 mm and lined with sawdust. The housing facility at Gyeongsang National University in Jinju, Korea, maintained a controlled environment with temperatures ranging from 20.7 to 21.2℃, relative humidity between 49.9 and 54.2%, ventilation at 10 to 15 cycles per hour, and 12 h of light daily (8 am to 8 pm) at an illumination intensity of 200 to 300 Lux. The mice had ad libitum access to food and water during the acclimatization and experimental periods. Environmental conditions, including temperature and humidity, were monitored every 30 min using automatic devices, and illuminance was regularly checked to ensure consistency.

Before the start of the experiment, all mice were acclimatized for one week to a standard chow diet (Harlan Laboratories Inc., Product No. 2018S; 12% fat, 63%

carbohydrates, and 25% protein). To induce obesity, mice were fed a high-fat diet (Research Diets Inc., D12492; 60% fat, 20% carbohydrates, and 20% protein). The experimental groups were as follows: normal diet + water (ND); high-fat diet + water (HFD); high-fat diet + green tea (GT); high-fat diet + fermented tea (FT); and high-fat diet + GABA tea (GBT). Over 13 weeks, mice that were fed a high-fat diet were administered tea extracts dissolved in physiological saline twice daily at a dose of 7.5 mL/kg. All animal welfare and experimental procedures complied with the National Institutes of Health guidelines for the care and use of laboratory animals (NIH Publication No. 8023, revised 1978). The study protocol was approved by the Animal Laboratory of the Gyeongsang National University (GNU-190924- M0044).

Monitoring of body weight and nutritional intake in mice

The body weights of the mice were precisely measured using a digital scale at baseline prior to the introduction of the tea extracts and subsequently every two weeks throughout the 13-week study. Food intake was meticulously tracked every weekly basis by weighing the food provided to each mouse at the start of the week and then again at the week's end.

Quantification of adipose tissue mass

Following extraction, adipose tissues were collected from multiple anatomical locations: left and right epididymal and both left and right perirenal areas, as well as brown fat deposits. Each sample of tissue was promptly rinsed with physiological saline to remove any blood and contaminants. After the initial rinse, the tissues were gently blotted with filter paper to remove excess moisture, ensuring they remained intact and undamaged during the process. Subsequently, the tissues were carefully laid out on a clean, dry surface for a brief period to allow any residual saline to evaporate naturally without affecting the tissue integrity. Once adequately dried, each adipose tissue sample was precisely weighed using an analytical balance to determine its mass.

Histological analysis of hepatic lipid accumulation

Oil Red O (ORO) staining was employed as the standard method for detecting neutral lipids and triglycerides in frozen liver sections, aiding in the identification of lipid accumulation within liver tissues [23]. Initially, liver tissues were frozen and sectioned into thin slices prepared for staining. These slices were subsequently fixed in a formalin solution to preserve tissue architecture. Following fixation, the sections were stained with ORO, a lipid-soluble dye targeting neutral lipids and triglycerides, which renders lipid droplets visibly red under a microscope. The stained tissue sections were then covered with glycerol gel for imaging and observed at 200× magnification. A minimum of five images per section were randomly captured to ensure representative sampling. Quantification of the ORO-stained areas was performed using the Columbus Image Data Storage and Analysis System (PerkinElmer, USA), employing an algorithm specifically designed for quantitative analysis of ORO-stained sections. This included intensity thresholding for image segmentation to distinguish the tissue from the background and accurately identify the ORO-stained lipid droplets within the sections.

Measurement of the serum metabolic parameters

Blood samples were collected from mice anesthetized with Avertin (seven mice per group), via the apex of the left ventricle using a 1-mL syringe. After collection, the samples were centrifuged at 900×*g* for 20 min to facilitate serum separation. The concentrations of serum triglycerides (TG), total cholesterol (TC), free fatty acids, aspartate aminotransferase (AST), and insulin were analyzed using enzymatic colorimetric assays, with the procedures conducted by Green Cross Reference Laboratory (Yongin-si, South Korea). The specific analysis codes employed were as follows: TG: C122, TC: C119, free fatty acids: C166, AST: C150, and insulin: E441. In addition, serum leptin levels were quantified employing mouse-specific immunoassay kits (catalog number MOB00B, R&D Systems, Minneapolis, MN, USA). All experimental procedures were performed in strict accordance with the manufacturer's protocols.

Statistical analysis

Statistical analyses were conducted using the GraphPad Prism 7 software (GraphPad Software, La Jolla, CA, USA). Data are presented as means ± standard error of the mean. We employed one-way ANOVA, the least significant difference test, and Student's t-test for statistical evaluations.

Results

Phenolic composition and antioxidant profiling of tea extracts

We analyzed the content of GABA, theanine, caffeine, vitamin C, EGCG, EGC, ECG, EC, and total catechins in GT, FT, and GBT (Fig. 1A and B). GT contained the highest levels of theanine, caffeine, vitamin C, EGCG, ECG, and total catechins, whereas GBT was abundant in GABA, EGC, and EC. Fermented tea had comparatively lower polyphenol content. The quantitative assessment of total phenolic content, denominated in gallic acid equivalents (GAE) per gram of dry extract, exhibited marked disparities across the examined tea varieties, with concentrations spanning from 16.30 to 167.67 mg GAE/g

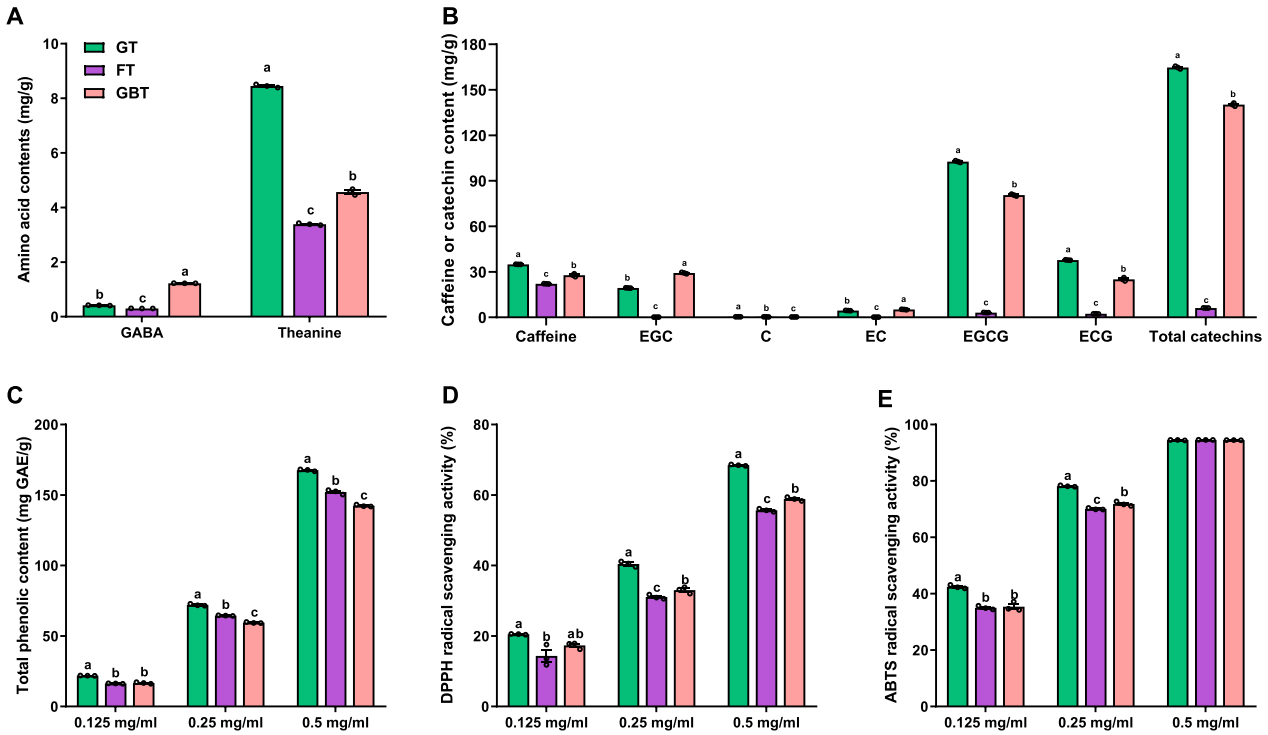

Fig. 1 Evaluation of polyphenol profiles and antioxidant capabilities in GT, FT, and GBT. **A** Content of GABA and L-theanine in the extracts. **B** Histograms comparing levels of key secondary metabolites across the tea types. **C** Total phenolic content of each tea extract quantified as milligrams of gallic acid equivalents per gram of extract. **D** Efficiency of the tea extracts in neutralizing DPPH free radicals. **E** Ability of the tea extracts to scavenge ABTS radicals was measured across different concentrations. *GABA* γ-aminobutyric acid, *EGC* epigallocatechin, *C* vitamin C, *EC* epicatechin, *EGCG* epigallocatechin-3-gallate, *ECG* epicatechin-3-gallate, *GAE* gallic acid equivalent, *DPPH* 2,2-diphenyl-1-picrylhydrazyl, *ABTS* 2,2′-azino-bis[3-ethylbenzothiazoline-6-sulfonic acid

(Fig. 1C). These findings indicate a substantial divergence in phenolic profiles attributable to the tea type and its respective processing technique. Furthermore, the antioxidant capacities of the tea extracts were rigorously determined via DPPH and ABTS assays, elucidating their free radical scavenging efficacy (Fig. 1D and E). GT manifested the most pronounced antioxidant activity, escalating with concentration. Sequentially, FT demonstrated considerable radical neutralizing capacity, with GBT displaying pronounced effectiveness in DPPH radical scavenging. Synthesizing these results delineates the distinct phenolic and antioxidant signatures of the tea extracts, underscoring the influence of their unique production methods. The elucidated data not only alludes to the varied health-promoting attributes inherent to each tea type but also accentuates the significance of processing techniques in shaping the bioactive properties of tea extracts.

Effects of functional tea extracts on body weight and food efficiency

We established an obese mouse model by feeding mice with an HFD for 13 weeks. The average body weight of mice in the HFD group was 45.16 g, which was significantly higher (18.02 g) than that of the 27.14 g in the ND group. During the 13 weeks, the body weight of the ND group increased by 10.16 g, whereas it increased by a much larger margin, 26.73 g, in the HFD group (Fig. 2A). In contrast, the GT, FT, and GBT groups showed lesser weight gains of 22.89 g, 20.1 g, and 21.46 g, respectively (Fig. 2B), amounting to 75.2% to 85.6% of the weight gain seen in the HFD group. GT2 was particularly effective at curbing weight gain.

Despite lower food consumption in the HFD group than in the ND group, the HFD group exhibited higher dietary efficiency (Fig. 2C; Table 1). The daily dietary intake was 2.02 g in the GT group and 1.19 g in the FT group, marginally less than the 2.11 g in the HFD group. Dietary efficiency decreased by 12.46% in the GT group, 11.09% in the FT group, and 10.73% in the GBT group, compared with 13.94% in the HFD group (Fig. 2D). Notably, the GBT group showed a significant inhibitory effect on weight gain, despite a 10.6% increase in dietary intake compared with that of the FT group and demonstrated lower dietary efficiency. These results indicate that all three tea extracts have

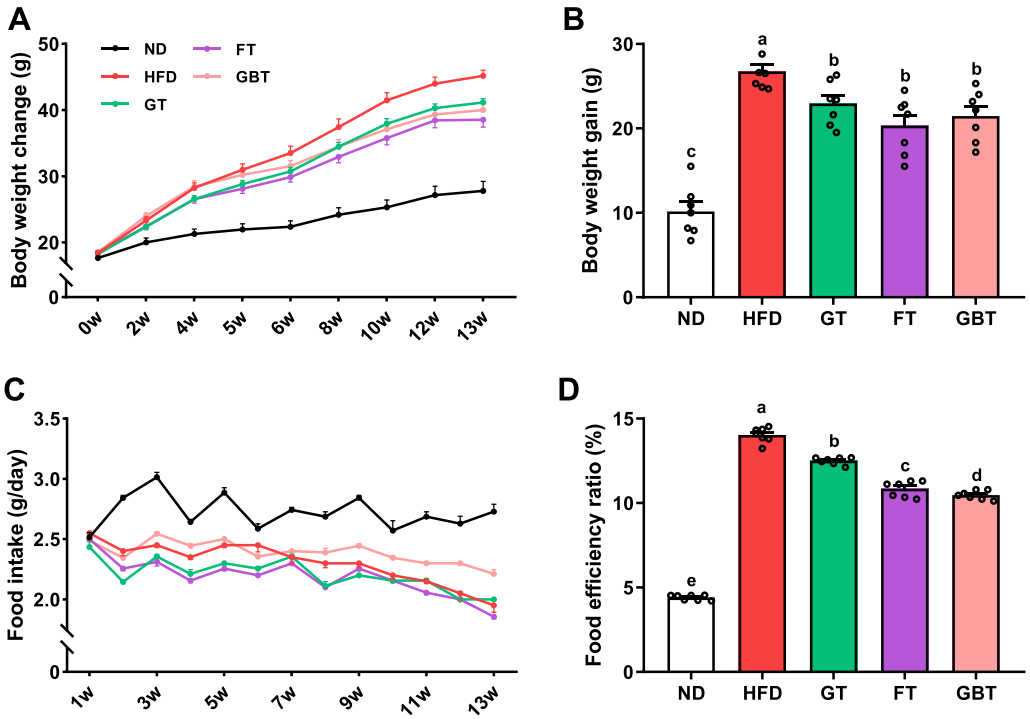

Fig. 2 Effects of GT, FT, and GBT extracts on high-fat diet mice model. **A** Average body weight change for 13 weeks. **B** The final body weight gain comparison across groups, **C** weekly monitored food intake, and **D** food efficiency ratio in obese mice treated with GT, FT, or GBT extracts. GT, FT, or GBT extracts have been found to reduce obesity markers in mice that are fed a high-fat diet, with GABA tea being particularly effective. *ND* normal diet, *HFD* high-fat diet, *GT* green tea, *FT* fermented tea, *GBT* GABA tea, *GABA* γ-aminobutyric acid

Table 1 Effects of green, fermented, and GABA tea extracts on body weight gain, food intake, and food efficiency ratio in high-fat diet-fed mice. GABA, γ-aminobutyric acid

Group	Body weight gains		Food intake (g/day)	Food efficiency ratio (%)[1]
	28 days (g)	g/day		
ND	10.16 ± 1.12	0.11 ± 0.01	2.53 ± 0.03	4.42 ± 0.50
HFD	26.73 ± 0.80$^{\#\#}$	0.29 ± 0.01$^{\#\#}$	2.11 ± 0.02$^{\#\#}$	13.94 ± 0.36$^{\#\#}$
GT	22.89 ± 0.85**	0.25 ± 0.01**	2.02 ± 0.01**	12.46 ± 0.41*
FT	20.10 ± 1.14**	0.22 ± 0.01**	1.99 ± 0.01**	11.09 ± 0.61**
GBT	21.46 ± 0.99**	0.24 ± 0.01**	2.20 ± 0.01**	10.73 ± 0.49**

Values are presented as mean ± standard error of the mean. #$p < 0.05$ and ##$p < 0.01$ compared to ND. *$p < 0.05$ and **$p < 0.01$ compared to HFD. [1]Food efficiency ratio = body weight gain per day

the potential to aid weight loss and enhance metabolic efficiency in a high-fat diet-induced obesity model.

Anti-obesity effects of GT, FT, and GBT

This study confirmed that mice on an HFD developed obesity, as indicated by increased body size compared with that in the ND group. In contrast, the groups treated with GT, FT, and GBT showed a significant reduction in body size relative to that of the HFD group (Fig. 3A). Specifically, the body weight in the HFD group more than doubled compared with that in the ND group (Fig. 3B), and liver weights increased from 0.96 to 1.44 g. Conversely, GT, FT, and GBT treatments reduced liver weights to 1.14 g, 1.08 g, and 1.12 g, respectively (Fig. 3C), demonstrating a protective effect against liver weight gain induced by HFD.

Furthermore, epididymal fat in the HFD group increased dramatically to 2.69 g, a 266% increase from 1.01 g in the ND group. This gain was effectively mitigated in the tea-treated groups, with GT and GBT showing a significant reduction of 23.9% in epididymal fat (Fig. 3D). Perirenal fat in the HFD group also surged, increasing by 372.9% to 1.46 g compared with 0.39 g in the ND group. Administration of functional tea extracts led to a marked decrease in perirenal fat in the GT, FT, and GBT groups (Fig. 3E), highlighting their efficacy in reducing fat accumulation, especially in the abdominal regions.

Reduction of lipid accumulation by GT, FT, and GBT

Histopathological examination of liver tissues after the 13-week dietary intervention revealed distinct differences among the groups. The ND group maintained a typical liver morphology. In contrast, the HFD group

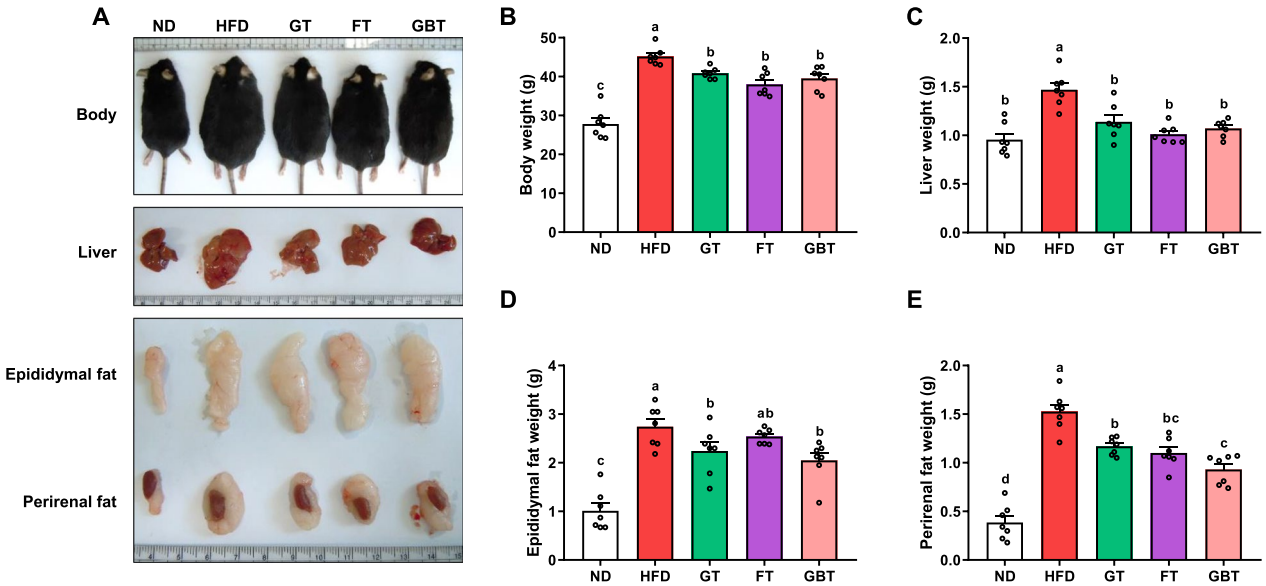

Fig. 3 Anti-obesity effect of GT, FT, and GBT on high-fat diet mice model. **A** Photographic representation of body size, liver, epididymal fat, and perirenal fat across five different groups: ND, HFD, GT, FT, and GBT. Bar graph depicting the **B** body weight of mice, **C** liver weight, **D** weight of epididymal fat, and **E** weight of perirenal fat, illustrating the impact of each dietary treatment. *ND* normal diet, *HFD* high-fat diet, *GT* green tea, *FT* fermented tea, *GBT* GABA tea, *GABA* γ-aminobutyric acid

exhibited significant fat accumulation, characterized by foam cell formation, oversized lipid droplets, and cellular necrosis. Additionally, this group exhibited signs of inflammatory cell infiltration. In contrast, the GT, FT, and GBT groups displayed reduced droplet-type fat accumulation; however, some inflammatory and Kupffer cell activation was observed (Fig. 4A). These findings indicate that functional tea extracts effectively mitigate the liver damage typically associated with a high-fat diet.

The extent of lipid deposition was further quantified using ORO staining, highlighting the size and distribution of fat granules in liver tissues (Fig. 4B). The livers from the HFD group showed classic indicators of fatty liver disease, as evidenced by a substantial number of lipid droplets. In contrast, the livers from the GT, FT, and GBT groups exhibited a notable decrease in lipid storage, underscoring the efficacy of these tea extracts in reducing the histological markers of fatty liver disease.

Effects of GT, FT, and GBT on obesity-related biochemical markers

We evaluated the effects of GT, FT, and GBT on serum lipid profiles and other biochemical markers associated with obesity in mice. In the ND group, the TG levels were measured at 69.7 mg/dL, whereas in the HFD group, these levels decreased to 55.0 mg/dL. The GT, FT, and GBT groups showed TG levels of 60.1 mg/dL, 58.9 mg/dL, and 55.8 mg/dL, respectively, with no statistically significant difference compared with that of the HFD group (Fig. 5A).

Serum TC levels were significantly higher in the HFD group than in the ND group (173 mg/dL). However, the tea extract-treated groups exhibited lower total cholesterol levels: 151.1 mg/dL in the GT, 141.3 mg/dL in FT, and 127.3 mg/dL in GBT (Fig. 5B). Free fatty acid concentrations were lower in all groups than in the ND group, but these differences were not statistically significant (Fig. 5C).

Liver function, as assessed by AST activity, increased in the HFD group, but the difference was not statistically significant. The FT and GBT groups showed a downward trend compared with that in the HFD group, indicating that the tea extracts did not adversely affect liver function (Fig. 5D). Serum leptin levels, significantly elevated in the HFD group at 38.9 ng/mL compared with 5.74 ng/mL in the ND group, decreased in the tea extract groups to 31.8 ng/mL in GT, 27.98 ng/mL in FT, and 27.36 ng/mL in GBT, reflecting a significant reduction in leptin concentration (Fig. 5E). Similarly, insulin levels, which rose in the HFD group to 0.41 ng/mL from 0.19 ng/mL in the ND group, were notably lower in the tea extract groups: 0.28 ng/mL in GT, 0.25 ng/mL in FT, and 0.30 ng/mL in GBT. This suggests that tea extracts exert an inhibitory effect on the serum insulin increase induced by a high-fat diet (Fig. 5F).

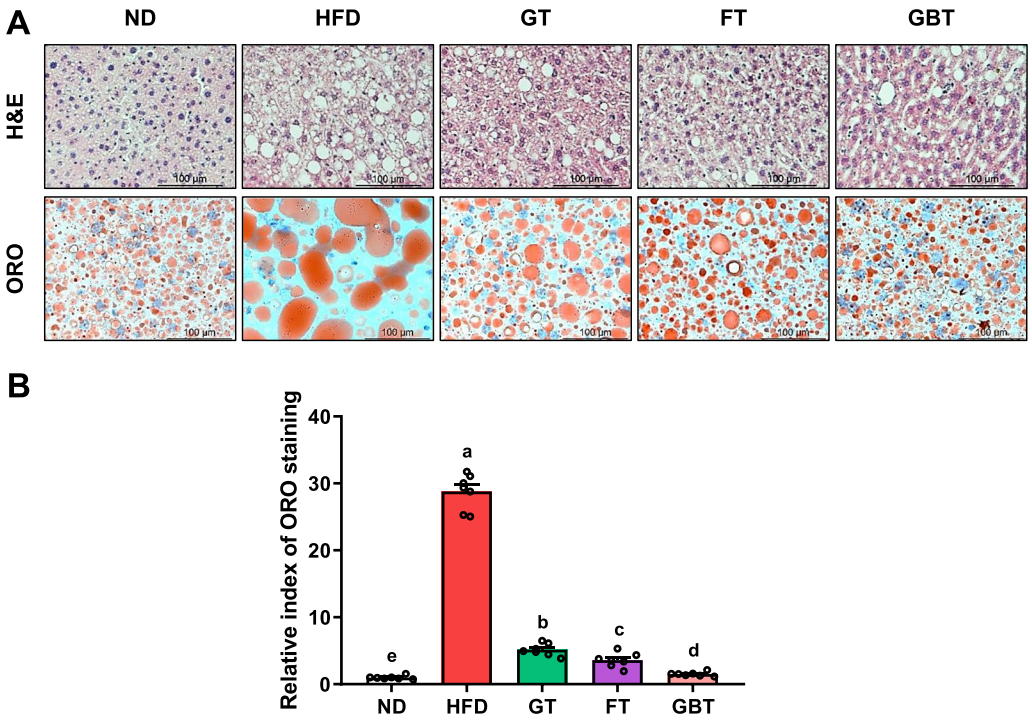

Fig. 4 Function tea extracts effectively reduce liver fat accumulation from a high-fat diet. **A** Images display H&E and ORO-stained liver sections from ND, HFD, GT, FT, and GBT groups. The H&E stain reveals cellular structure, while ORO specifically highlights lipid droplets characteristic of fatty liver or steatosis. **B** The accompanying bar graph quantifies lipid droplet size in these groups based on ORO staining intensity, showcasing the varying degrees of lipid accumulation with statistical differences marked by letters. This suggests the effectiveness of GT, FT, and GBT extracts in reducing liver fat accumulation induced by a high-fat diet. *ND* normal diet, *HFD* high-fat diet, *GT* green tea, *FT* fermented tea, *GBT* GABA tea, *GABA* γ-aminobutyric acid, *H&E* Hematoxylin and Eosin, *ORO* Oil red O staining

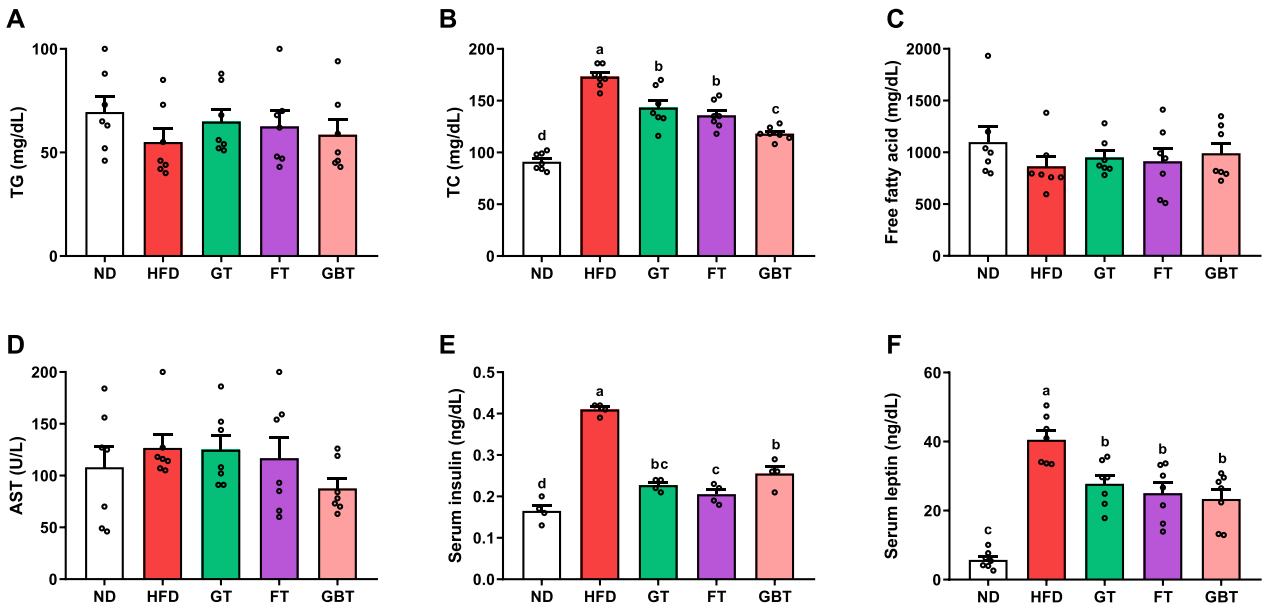

Fig. 5 Effect of GT, FT, and GBT on obesity-related biochemical markers. The graphs detail the influence of GT, FT, or GBT extracts on **A** TG levels, **B** TC levels, **C** free fatty acid concentrations, **D** AST activity as a liver function marker, **E** serum leptin levels associated with fat mass, and **F** serum insulin levels related to glucose metabolism. GT, FT, and GBT appear to mitigate obesity-related biochemical markers in mice on a high-fat diet. *ND* normal diet, *HFD* high-fat diet, *GT* green tea, *FT* fermented tea, *GBT* GABA tea, *GABA* γ-aminobutyric acid, *TG* triglyceride, *TC* total cholesterol, *AST* aspartate aminotransferase

Effects of GT, FT, and GBT on fasting blood glucose regulation.

Considering previous findings that obesity negatively affects glucose metabolism, our study explored the potential of GT, FT, and GBT, known for their anti-obesity properties, to enhance glucose regulation. In both the ND and HFD groups, the fasting blood glucose levels progressively increased throughout the experiment. However, the groups treated with GT, FT, and GBT displayed a decrease in fasting blood glucose levels as the weeks advanced (Fig. 6A).

After 13 weeks, the fasting blood glucose levels in the HFD group were significantly higher than those in the ND group. Notably, the GT, FT, and GBT groups showed a marked reduction in fasting blood glucose levels (Fig. 6B).

Discussion

As obesity rates escalate globally, the consequent increase in related health conditions has sparked intense interest in dietary interventions with anti-obesity effects [24]. The most consumed varieties of tea, green (unfermented) black (fully fermented), and GABA, are derived from Camellia sinensis leaves. These teas are rich in phytochemicals, such as polyphenols, pigments, polysaccharides, alkaloids, free amino acids, and saponins [25]. These teas go beyond their conventional uses and offer a spectrum of bioactive benefits, including antioxidant, anti-inflammatory, antibacterial, anticarcinogenic, anti-hypertensive, neuroprotective, cholesterol-lowering, and thermogenic effects [26]. Moreover, they exhibit prebiotic-like qualities that further enhance their potential as health-promoting agents.

This study evaluated the anti-obesity effects of GT, FT, or GBT in a high-fat diet-induced mouse obesity model. The research entailed administering these tea extracts—standard GT, FT, and GBT—at 7.5 mL/kg doses twice daily for 13 weeks following obesity induction. We assessed numerous factors, including body weight, food intake, blood lipid profiles, abdominal adipose tissue, and several obesity-related biochemical markers. We also conducted a comprehensive analysis of both morphological and biochemical liver function indicators [27]. Tea extracts were found to effectively reduce body weight in high-fat diet-induced obesity, likely due to their influence on reducing dietary efficiency. Notably, the extended consumption of tea extracts, particularly GABA, was more effective in mitigating weight gain.

Typically, a high-fat diet is associated with increased concentrations of neutral lipids compared with a normal diet [28]. In contrast, our study yielded unexpected results. The high-fat diet used in our research contained 30% lard, which coincided with a reduction in serum TG levels [29], suggesting that the type of dietary fat influences triglyceride storage differently. Excessive energy accumulation boosts leptin production, which is closely associated with obesity, body mass, and fat content [30]. The decrease in serum leptin levels after the administration of the GT, FT, and GBT resulted in a reduction in body weight and adipose tissue. Likewise, serum insulin levels, which typically escalate with body mass and visceral fat accumulation, were reduced after treatment with tea extracts, presumably due to weight reduction and diminished visceral fat [31].

The detrimental effects of obesity on health are well documented [32]. Our study aimed to determine whether GT, FT, and GBT, which are effective against obesity, could ameliorate glucose metabolism. In both the normal and high-fat diet groups, fasting blood glucose levels tended to increase with age. Conversely, in mice treated with GT, FT, and GBT, a decline in fasting blood glucose levels was noted over time, indicating a role for the extracts in obesity management through improved glucose metabolism.

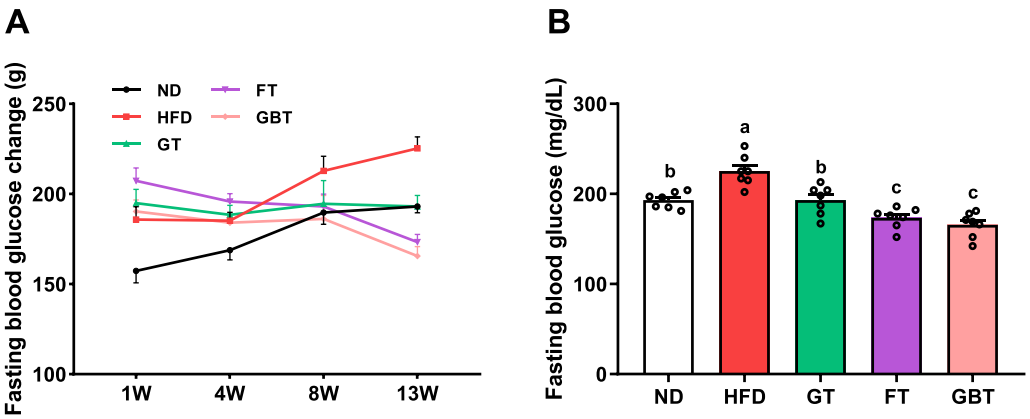

Fig. 6 GT, FT, and GBT regulate glucose metabolism in mice. **A** Trends in fasting blood glucose over 13 weeks for ND, HFD, and tea extracts-treated (GT, FT, GBT) diets. **B** Bar graph of fasting glucose levels after 13 weeks, showing significant reductions in the tea-treated groups. *ND* normal diet, *HFD* high-fat diet, *GT* green tea, *FT* fermented tea, *GBT* GABA tea, *GABA* γ-aminobutyric acid

Furthermore, we assessed the liver function to monitor the potential side effects of the extracts. AST levels, an indicator of liver health, showed no significant increase in the tea extract-treated groups compared with that in the high-fat diet group, implying that the extracts did not negatively affect liver function [33].

In our study, we observed that samples of GT, FT, and GBT exhibited high levels of theanine, caffeine, and GABA. These compounds are noted in the literature for their potential benefits in weight management and metabolic health [33]. Theanine, an amino acid present in tea leaves, has been associated with reduced stress and improved metabolic function, both factors that may indirectly mitigate obesity [34]. Caffeine, recognized for stimulating metabolism, is linked to increased energy expenditure and fat oxidation [35]. GABA tea demonstrated pronounced anti-obesity effects that may be attributed to its elevated GABA content, known to enhance antioxidant activity. Consistent with recent studies, GABA has been shown to limit weight gain and reduce fat accumulation through various mechanisms, including anti-inflammatory and antioxidative effects, improved glucose metabolism, β-cell function modulation, ketogenesis stimulation, and increased circulating levels of GABA and β-aminoisobutyric acid [36–38]. The interplay between these bioactive compounds in tea illustrates their collective impact on metabolic rate, stress response, and lipid metabolism. The synergistic effect of theanine, caffeine, and GABA in tea may provide a holistic anti-obesity action [25], indicating that tea consumption could offer a multifaceted strategy for managing weight.

In conclusion, this research elucidates the robust capacity of GT, FT, and GBT to mitigate metabolic imbalances induced by a high-fat diet, as graphically illustrated in Fig. 7. Over 13 weeks, mice on a high-fat regimen exhibited marked increases in body weight, and

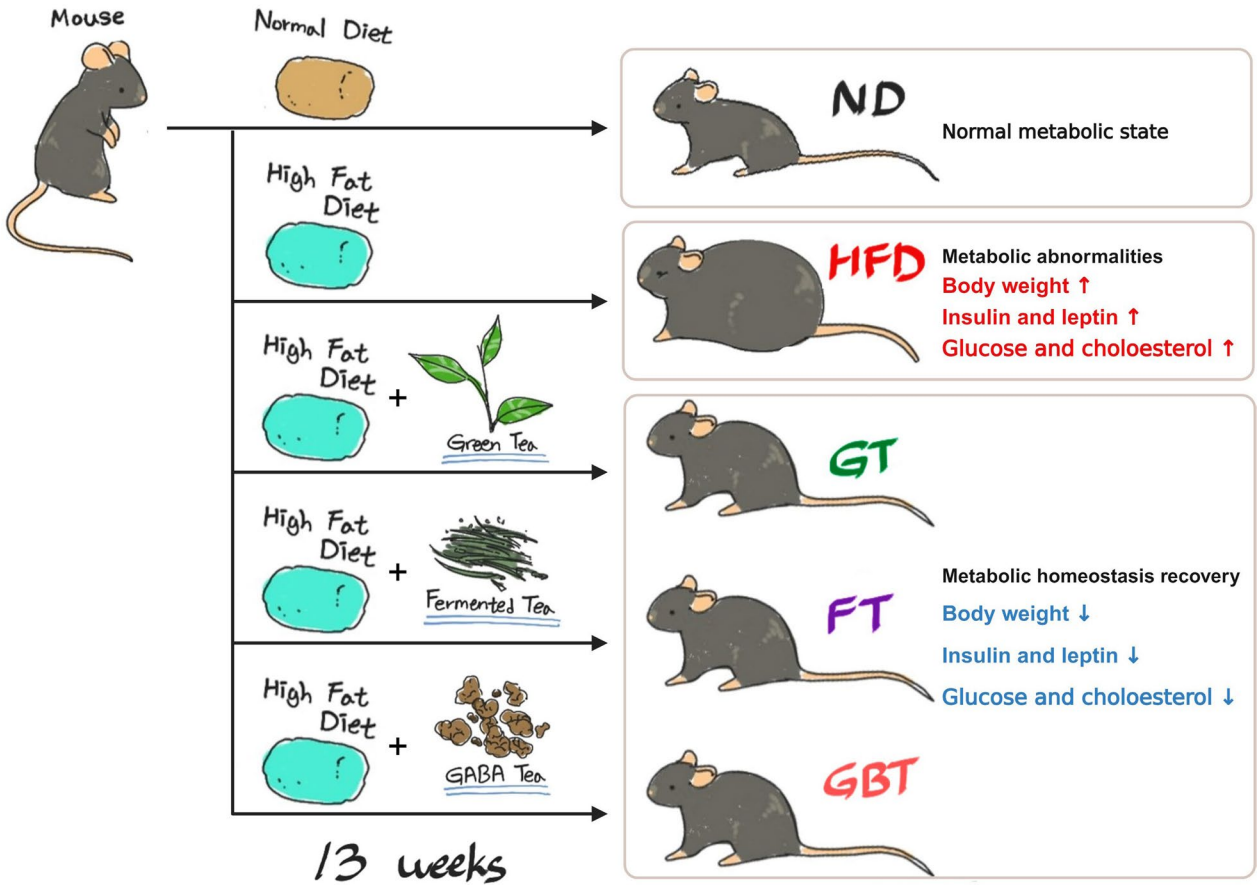

Fig. 7 Comparative effects of GT, FT, and GBT supplementation on high-fat diet-induced obesity mouse model. This schematic diagram depicts the results from a 13-week study on the influence of GT, FT, or GBT on metabolic health indicators in an obesity model induced by a high-fat diet. While the ND group shows a normal metabolic state, mice on the HFD exhibit significant metabolic disruptions, including increased body weight, insulin, leptin, glucose, and cholesterol levels. Supplementation with GT, FT, or GBT leads to an observable shift towards metabolic equilibrium, marked by reduced body weight and lower levels of insulin, leptin, glucose, and cholesterol, highlighting the potential of these tea supplements in counteracting obesity-related metabolic imbalances. *ND* normal diet, *HFD* high-fat diet, *GT* green tea, *FT* fermented tea, *GBT* GABA tea, *GABA* γ-aminobutyric acid

levels of insulin, leptin, glucose, and cholesterol, signaling metabolic disruption. In contrast, the introduction of GT, FT, and GBT not only attenuated weight gain but also realigned metabolic parameters, highlighting their restorative influence on metabolic homeostasis. These tea extracts prove to be potent agents for weight management and metabolic health enhancement, with their non-toxic liver profile reinforcing their safety for regular consumption and their integral role in health promotion strategies. The consolidation of these outcomes emphasizes the dual functionality of the tea extracts as holistic dietary supplements for metabolic health improvement and lays a solid foundation for future advancements in tea production technology and comprehensive safety assessments.

Abbreviations

EGCG	Epigallocatechin-3-gallate
GABA	γ-Aminobutyric acid
ECG	Epicatechin-3-gallate
EGC	Epigallocatechin
EC	Epicatechin
C	Vitamin C
ND	Normal diet with water
HFD	High-fat diet with water
GT	Green tea
FT	Fermented tea
GBT	GABA tea.
AST	Aspartate aminotransferase
ORO	Oil Red O
DPPH	2,2-Diphenyl-1-picrylhydrazyl
ABTS	2,2′-Casino-bis [3-ethylbenzothiazoline-6-sulfonic acid
ANOVA	ANalysis Of Variance
TG	Triglyceride
TC	Total cholesterol

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Author contributions
Conceptualization, Y-S.H: methodology, S-J.L: software, J-Y.H: validation, K.H.C: formal analysis, J.C.K: investigation, H.C.C: resources, H.J.C: data curation, J-Y.H: writing—original draft preparation, S-J.L: writing—review and editing, Y-S.H: visualization, S-J.L: supervision, Y-S.H: project administration, Y-S.H: funding acquisition, H.C.C. All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials
The data presented in this study are available on request from the corresponding author.

Declarations

Competing interests
The authors declares that they have no competing interests.

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