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# Theabrownins from dark tea: formation, gut microbiota-mediated biotransformation, health benefits, and potential in functional food applications

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Theabrownins (TBs) are the major polyphenol-derived pigments and key bioactive components in dark tea formed through microbial fermentation or enzymatic oxidation. This review provides a comprehensive overview of the formation pathways, structural complexity, and diverse biological functions of TBs, highlighting recent advances in their production and application. Emerging evidence indicates that TBs exert significant health-promoting effects, including anti-obesity, anti-diabetic, anti-inflammatory, antioxidant, anti-cancer, anti-photodamage, and gut microbiota-modulating activities. These effects are mediated through multiple molecular mechanisms involving the regulation of bile acid metabolism, intestinal microbial composition, circadian rhythm genes, and signaling pathways such as AMPK/PGC1 $\alpha$ , PI3K/Akt, FoxO/PPAR, FXR/FGF15, NRF2, and NF- $\kappa$ B. Additionally, TBs influence apoptosis, autophagy, and epigenetic modifications, thereby further contributing to their therapeutic potential. Despite promising bioactivities, challenges remain in elucidating TBs' precise molecular structures, optimizing large-scale production, and translating findings from cell and animal studies into human clinical trials. Addressing these limitations is critical for advancing TBs from functional components to evidence-based nutraceutical ingredients. Overall, this review summarizes current knowledge on TBs' bioactivities and underlying mechanisms. It also provides insights for future research and industrial development, supporting the potential of TBs as natural agents for disease prevention and human health promotion.

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## 1. Introduction

Tea is the second most widely consumed beverage globally, following only water. Based on the processing technique and fermentation degree, tea can be classified into six major categories: green tea (unfermented), white tea, yellow tea, oolong tea (semi-fermented), black tea (fully fermented), and dark tea

(post-fermented).<sup>1</sup> Each type of tea possesses unique characteristics in infusion color, aroma, flavor, and taste. Among these types of tea, dark tea (also known as Hei Cha) originated in China and can be traced back to the Tang dynasty (733–804 AD).<sup>2</sup> Its development is historically linked to the Cha Ma Road trade routes, where prolonged transportation under humid conditions inadvertently catalyzed microbial fermentation. Today, popular types of dark tea include ripened Pu-erh tea (Yunnan), Liubao tea (Guangxi), Fuzhuan brick tea (Hunan and Shaanxi), Qingzhuan brick tea (Hubei), and Kangzhuan brick tea (Sichuan) from different provinces (Fig. 1).<sup>3</sup> Beyond sensory properties, tea consumption has been associated with various health benefits, including reducing obesity and cardiovascular diseases, as well as protective effects against certain cancers and neurodegenerative diseases.<sup>4</sup> Numerous studies have reported that fermented teas exhibit superior hypolipidemic effects and greater efficacy in regulating lipid metabolism compared to unfermented teas.<sup>5–7</sup> These biological activities are largely attributed to a group of water-soluble pigments, including theaflavins (TFs), thearubigins (TRs), and theabrownins (TBs) produced during fermentation.

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**Fig. 1** Representative types of commercial dark tea products, including ripened Pu-erh tea, Liubao tea, Qingzhuang brick tea, Kangzhuang brick tea, and Fuzhuang brick tea.

TBs represent the major water-soluble brown pigments in dark teas, accounting for approximately 10–12% of the tea's dry weight,<sup>8</sup> whereas TFs and TRs are more abundant in black teas.<sup>9</sup> Numerous *in vitro* and *in vivo* studies have shown that TBs may confer a variety of health-promoting activities, including anti-obesity,<sup>10</sup> anti-cancer,<sup>11</sup> anti-inflammation,<sup>12</sup> uric acid-lowering,<sup>13</sup> and lipids-lowering, supporting their promising potentials in human health promotion. Moreover, several studies have explored the molecular mechanisms underlying these effects.<sup>14,15</sup> Despite these promising findings, only a limited number of review articles have comprehensively summarized the formation, chemical properties, and health-promoting bioactivities of TBs.<sup>16,17</sup> Notably, the role of TBs in modulating gut microbiota, an emerging area that is critical to metabolic and systemic health, remains insufficiently investigated. Herein, we aim to provide an updated and systematic account of the research in TBs, emphasizing their biological activities, especially in metabolic syndrome and associated diseases. We highlight recent discoveries on the protective effects of TBs against obesity, lipid disorders, inflammation, and tumorigenesis, alongside detailed discussions of the underlying molecular mechanisms. Special attention is given to the emerging evidence linking TBs to gut microbiota modulation and its implications for health benefits. Through this work, we seek to facilitate theoretical advancements and to support future industrial applications of TBs and fermented dark teas.

## 2. Chemical properties of TBs

TFs, TRs, and TBs are the three major types of water-soluble pigments produced during tea fermentation.<sup>18</sup> Among them, TBs are heterogeneous, water-soluble pigments characterized by brown or reddish-brown color. TBs are the most abundant group of phenolic pigments in black teas, accounting for approximately 60% of the solids in a typical black tea infusion, and are considered as one of the most critical components for evaluating the quality of dark tea.<sup>19</sup> While the structures of TFs and TRs have been extensively studied,<sup>20,21</sup> the structural elucidation of TBs remains challenging.

### 2.1 Molecular weight distribution and characteristics

TBs are macromolecules with a wide molecular weight distribution ranging from 3.5 kDa to more than 100 kDa, with an

average molecular weight of *ca.* 6200 Da.<sup>22</sup> This molecular diversity contributes to the complexity and heterogeneity of TBs. Detailed analyses using combustion analysis, infrared spectroscopy, and nuclear magnetic resonance (NMR) spectroscopy have revealed that TBs are prone to concentration-driven aggregation in aqueous solution and retain the chiral properties of flavanols and TFs.<sup>19</sup> Fractionation studies have further demonstrated structural diversity within TBs. For instance, it was demonstrated that polysaccharide-rich TBs fractions from Fuzhuang brick tea can be categorized into five molecular weight groups as <3 kDa (59%), 3–10 kDa (21%), 10–30 kDa (12%), 30–100 kDa (6%), and >100 kDa (2%). All fractions exhibited characteristic UV-visible spectroscopic peaks around 204 nm and 274 nm, suggesting the presence of polyphenolic contents.<sup>23</sup> This is supported by the study of Zhang *et al.*, the three TBs prepared from enzymatic fermentation, alkaline oxidation, and Pu-erh tea showed similar UV-Vis absorption with the maximum at 205 nm and 270 nm, suggesting the appearance of aromatic compounds.<sup>24</sup> Additional compositional studies revealed that TBs from Zijuan tea contained a mixture of lipids, ketones, nitrogenous compounds, acids, hydrocarbons, phenols, alcohols, aldehydes, and aromatic compounds, with dominant fractions found both below 3.5 kDa and above 100 kDa.<sup>25</sup> The high molecular weight fractions (>100 kDa) were primarily composed of phenolic pigments, esters, proteins, and polysaccharides, while the low molecular weight fractions contained no detectable polysaccharides.

### 2.2 Structural analysis and chemical composition

Despite the inherent challenges, significant progress has been made toward revealing TBs complex chemistry through a combination of analytical techniques. Liquid chromatography-mass spectrometry (LC-MS) analyses have supported the hypothesis of the presence of polyhydroxylated TBs. This suggests that TBs are composed predominantly of polyhydroxylated catechin dimers (*e.g.*, TFs and their galloyl derivatives) in dynamic redox equilibrium with their associated quinones.<sup>26</sup> Mass spectrometry studies have identified over 5000 distinct chemical entities within TBs from green tea and black tea, capable of spontaneous nano-aggregate formation in aqueous media.<sup>27</sup> Curie-point pyrolysis-gas chromatography-mass studies have shown that TBs precursors include a wide array of



components such as catechins, tea pigments, caffeine, polysaccharides, proteins, and lipids.<sup>23</sup> Morphologically, TBs in the 10–30 kDa range exhibit amorphous, thermostable properties, with a slice-shaped smooth surface and island-like structure. Moreover, UV-Vis spectroscopy analyses have suggested the presence of abundant hydroxyl and carboxyl groups, as well as glycosidic bonds, illustrating extensive polymerization of phenolic compounds within TBs.<sup>28</sup> Advanced hydrolysis and compositional analyses of TBs from Tibetan tea using UHPLC-Q-TOF-MS revealed the presence of small molecules including jaceosidin, triethyl citrate,  $\alpha$ -naphthoflavone, epicatechin, voriconazole, quercetin, apigenin 7-rutinoside, and dodecylbenzenesulfonic acid, which were identified as major structural components.<sup>22</sup> These findings underscore the highly heterogeneous and polyphenol-rich nature of TBs and support their classification as a polymeric matrix composed of diverse flavonoids, phenolic acids, alkaloids, and aromatic derivatives. Additionally, different preparation processes and various derivations affect the structures and composition. Comparative studies have shown that TBs produced by enzymatic, alkaline, or traditional Pu-erh tea fermentation methods differ slightly in chemical profiles, with amino acids predominating in enzymatic and alkaline TBs, and polyphenols, caffeine, and flavonoids being more abundant in Pu-erh tea-derived TBs.<sup>24</sup> Although significant progress has been made, the precise molecular structures of TBs remain elusive. Given their chemical heterogeneity and the limitations of current analytical technologies, further efforts combining advanced structural techniques are warranted to fully elucidate the molecular characterization.

### 3. The formation of TBs

Tea fermentation plays a pivotal role in developing the distinctive chemical composition and sensory properties, contributing to the health benefits associated with dark tea consumption. In fresh tea leaves, eight major catechins are commonly identified, including catechin, gallic catechin (GC), catechin gallate (CG), gallic catechin gallate (GCG), epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG).<sup>29</sup> EGCG, ECG, and EGC are typically present in higher concentrations in the leaves of most tea cultivars.<sup>30,31</sup> Catechins in fresh tea leaves are the main precursor substances for TB formation.<sup>32</sup> Polyphenol oxidase (PPO) initiates the oxidation process by catalyzing the conversion of *o*-hydroxyphenol to *o*-quinone in the presence of oxygen.<sup>33,34</sup> These *o*-quinones subsequently undergo oxidative polymerization to form TFs and TRs, which further interact with other chemicals, including caffeine, lipids, proteins, and polysaccharides, to produce higher molecular weight polymers of TBs.<sup>35,36</sup> In addition, TBs can be directly formed by peroxidase (POD) that catalyzes quinones without necessarily passing through TFs and TRs intermediates. Studies have shown that during the fermentation process, the concentration of TBs and caffeine increases steadily, whereas the levels of free phenols

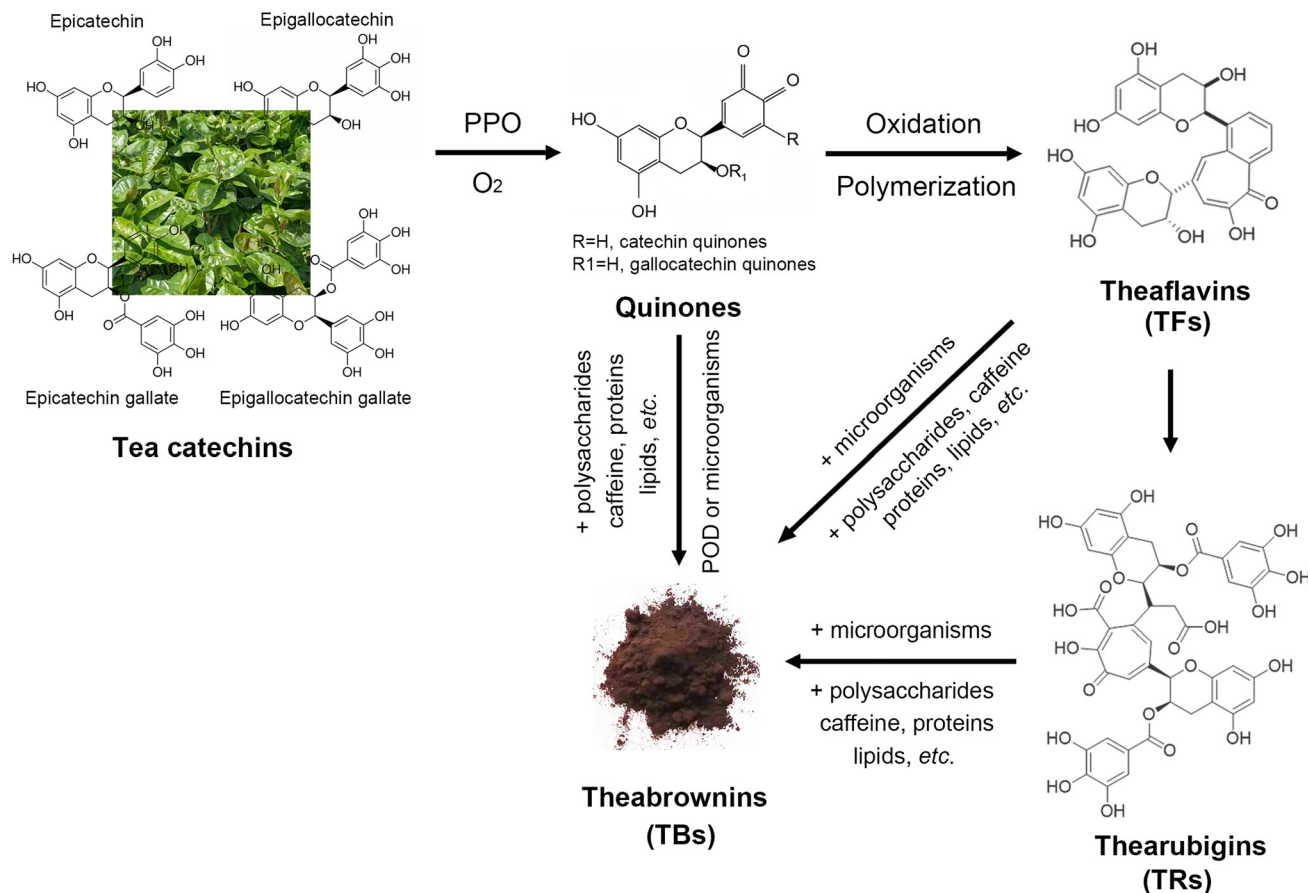
and amino acids decline, supporting the notion that TBs are formed from catechins *via* oxidation and polymerization reactions.<sup>37</sup> Furthermore, the activity of extracellular PPO and POD enzymes has been positively correlated with the accumulation of TBs,<sup>38</sup> underscoring the critical roles of these oxidation enzymes in the formation of TBs. Thus, it is inferred that tea catechins are either continuously oxidized and polymerized through quinone intermediates to form TFs and TRs, which then further react with other tea components (*e.g.*, polysaccharides, lipids, *etc.*) to produce TBs, or quinones may be polymerized to form TBs directly without intermediates (summarized in Fig. 2). However, due to the structural complexity and the enormous diversity of compounds involved, making the isolation and the characterization of TBs extremely challenging, the detailed mechanisms underlying TBs formation remain unclear. Currently, TB pigments can be produced from microbial fermentation or microbe-free methods.

#### 3.1 By microbial fermentation

The post-fermentation of dark tea, a spontaneous fermentation during storage, involves microbial activity that continues even after the initial oxidation of tea leaves, where microorganisms play critical roles. This process can last from several months to several years, contributing to the unique sensory profiles of dark tea.<sup>2</sup> For instance, during the pile-fermentation process, a critical step in the manufacture of dark tea that typically takes 1–2 months,<sup>39</sup> TBs are often generated and greatly impart the characteristic auburn-black color and mellow flavor of dark tea.<sup>40</sup>

In the characteristic solid-state fermentation condition, water activity remains below 0.7, favoring the growth of fungi and yeasts rather than bacteria. Various fungi and yeasts, including *Aspergillus* spp., *Rhizomucor* spp., *Penicillium* spp., and *Pichia* spp., have been isolated from fermented dark teas. These strains have been used in solid-state fermentation,<sup>41</sup> submerged fermentation,<sup>42</sup> and liquid-state fermentation<sup>15,43</sup> methods to produce high-yield and high-quality TBs for industrial applications. Among these fungi, *A. tubingensis*, *A. marvanovae*, *A. fumigatus*, *R. pusillus*, and *R. tauricus* isolated from solid-state fermentation have shown remarkable abilities in converting tea polyphenols into TBs.<sup>44</sup> *Aspergillus niger* inoculation involves in tea fermentation, accelerates the fermentation process and facilitates biotransformation of aroma and taste metabolites, which promoted TBs accumulation and improved the quality of dark tea.<sup>39</sup> The inoculum of *A. fumigatus* from *Aspergillus* spp., led to TBs production of 158 g kg<sup>-1</sup> of sun-dried green tea leaves after 6 days of fermentation at 45 °C, supporting the critical roles of *Aspergillus* spp. in TBs production during the pile-fermentation process.<sup>38</sup> Compared to solid-state fermentation, submerged fermentation has shown a higher yield of TBs, emerging as a widely adopted method for industrial-scale generation of TBs.<sup>45</sup> Submerged fermentation of sun-dried green tea infusions with the inoculum of *A. tubingensis* TISTR 3646, *A. tubingensis* TISTR 3647, *A. marvanovae* TISTR 3648, and *A. fumigatus* TISTR 3654 achieved the yields of TBs production to 6.5, 12.4, 11.1, and





**Fig. 2** Proposed formation pathway of TBs from tea polyphenols during fermentation. Tea catechins and other phenolics are first oxidized to quinones by polyphenol oxidase (PPO), which are subsequently polymerized into theaflavins (TFs), thearubigins (TRs), and theabrownins (TBs). Additionally, quinones may be directly converted into TBs through peroxidase (POD)-catalyzed polymerization. Abbreviations: PPO, polyphenol oxidase; POD, peroxidase.

8.4 g L<sup>-1</sup>, respectively.<sup>46</sup> Under optimized conditions for submerged fermentation, *i.e.*, 27.5 mL g<sup>-1</sup> liquid–solid ratio, 5% of *A. niger* inoculum, and 184 rpm rotation speed, TBs production reached to 291.9 g kg<sup>-1</sup> in instant dark tea.<sup>47</sup> The production rates of three *Aspergillus* species, including *A. cristatus*, *A. niger*, and *A. tubingensis*, were compared in liquid-state fermentation with instant dark tea. Fermentation with *A. tubingensis* exhibited the highest production of TBs, while *A. cristatus* fermentation resulted in the lowest content of TBs. These findings underscore the strong correlation between the dominant microbial species and the efficiency of TBs production in liquid-state fermentation.<sup>48</sup>

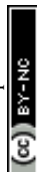
Apart from *Aspergillus* spp., microbial analyses during the manufacturing process of Fuzhuan brick tea identified fungi (*Aspergillus*, *Candida*, unclassified\_o\_Hypocreales, and *Wallemia*) and bacteria (*Klebsiella*), the core functional microorganisms associated with the metabolic variations.<sup>49</sup> Fuzhuan brick tea is characterized by a unique “golden flower blossoming” on its surface, primarily attributed to fermentation by *Eurotium cristatum*.<sup>50</sup> In an artificial solid-state fermentation, green tea leaves inoculated with an *E. cristatum* spores ( $2 \times 10^4$  CFU per g) at 28 °C and 70% humidity for 12 days, resulted in TBs production

reaching approximately 175 g kg<sup>-1</sup>.<sup>41</sup> These findings suggest that fungi, including *Aspergillus* spp. and *E. cristatum*, are essential in the efficient transformation of TBs from tea polyphenols. One recent publication showed that *Aspergillus* became prominent in the mid-fermentation stage, and *Brevibacterium* became dominant in the late-fermentation stage, and increased TBs content to 219.33 mg g<sup>-1</sup> in basket fermentation, suggesting that bacteria played an important role in later stages of tea fermentation.<sup>51</sup>

### 3.2 By microorganism-free reactions

Beyond traditional microbial fermentation, recent studies have also demonstrated the production of TBs through microorganism-free methods. These processes employ chemical or enzymatic oxidation systems to simulate the natural fermentation process, offering a more controlled approach to TBs production, encouraging the industrial generation and utilization of TBs in a new and hopeful approach.

(i) Enzymatic oxidation: in microorganism-free systems, green tea leaves or tea infusion are fermented using extracellular enzymes produced by the functional microbes. TBs conversion is highly correlated with the activity of the extracellular PPO and POD enzymes.<sup>38</sup> In solid-state fermentation, adding



PPO to sun-dried green tea leaves enhanced TBs production. Specifically, leaves with PPO at activities of 3920 U and 11 760 U achieved TBs production of 214.5 and 199.9 g kg<sup>-1</sup>, respectively, accompanied by a significant reduction in tea polyphenol content, thereby improving the sensory characteristics of the brewed liquor.<sup>52</sup> Enzymatic oxidation using PPO and POD enabled tea catechins to be successfully converted into TBs with a conversion rate of 91%, supporting the theory of the industrial-scale production of TBs from tea polyphenols.<sup>53</sup> In addition to PPO and POD, laccase has been recognized as another key extracellular enzyme involved in TBs formation.<sup>54</sup> A crude enzyme concentrate of *A. tubingensis* TISTR 3647, containing POD, PPO, and laccase, was shown to effectively oxidize tea phenolic substances into TBs when added to sun-dried green tea fusions.<sup>55</sup> Compared to microbial fermentation methods, enzymatic oxidation demonstrated significantly higher efficiency; the volumetric production rate of TBs *via* enzymatic fermentation was nearly 8-fold greater than that of solid-state fermentation and 24-fold higher than that of submerged fermentation, offering a more controlled and productive approach for commercial TBs production.<sup>55</sup>

(ii) Alkali oxidation: in addressing environmental concerns associated with the use of organic solvents during TBs extraction and purification, researchers have explored the production of TBs from tea polyphenols *via* weak alkali oxidation.<sup>56</sup> It is reported that tea catechins, when oxidized under conditions of weak alkalinity (pH 6.0, 35 °C) with sodium bicarbonate (NaHCO<sub>3</sub>) and PPO, were efficiently converted to TBs with a conversion efficiency of 96%. Similarly, another study applied NaHCO<sub>3</sub> (1.0 mg mL<sup>-1</sup>) to the tea polyphenols solution, followed by heating at 95 °C for 5 hours to initiate the oxidative polymerization, which resulted in a 95% conversion rate of TBs.<sup>57</sup> These findings suggest that weak alkali oxidation offers a promising, environmentally friendly strategy for industrial production of TBs from tea polyphenols.

## 4. Health-promoting effects of TBs

The consumption of dark tea has been associated with a wide range of health benefits, particularly in improving metabolic syndrome and its related conditions. As the principal bioactive components in dark tea, TBs exhibit diverse physiological activities that contribute to their health-promoting potential.<sup>58</sup> These effects include anti-obesity and anti-diabetic properties, antioxidant capacity, mitigation of fatty liver disease, attenuation of inflammation, regulation of circadian rhythm, modulation of intestinal microbiota flora, and regulation of bile acid (BA) metabolism. A summary of these biological functions is presented in Table 1. In the following sections, the major biological effects of TBs and their underlying molecular mechanisms are systematically reviewed.

### 4.1 Improvement of metabolic syndrome

The global prevalence of overweight and obesity (body mass index ≥25) continues to rise (World Obesity Atlas 2024).

Obesity is a major risk factor for fatty liver disease, diabetes, cardiovascular disease, neurodegenerative disease, and cancer.<sup>59,60</sup> According to the 2024 World Obesity Atlas, obesity is projected to cost the global economy over US\$ 4 trillion by 2035—approximately 3% of global GDP (World Obesity Atlas 2024). While conventional interventions such as dietary changes, physical activity, pharmacotherapy, and bariatric surgery are available, many anti-obesity drugs (*e.g.*, pancreatic lipase inhibitors or appetite suppressants) have limited efficacy and adverse effects.<sup>61</sup> Thus, there is a growing interest in safe, natural alternatives such as TBs with demonstrated potential to regulate glycolipid metabolism.<sup>62</sup>

**4.1.1 Anti-obesity and dyslipidemia.** Accumulating evidence highlights the anti-obesity effects of TBs through multiple mechanisms.<sup>10,63,64</sup> In a mouse model, 12 weeks of TBs intervention following a high-fat diet (HFD) pretreatment significantly reduced body weight gain, supporting the therapeutic roles in obesity management.<sup>63</sup> TBs from black tea at a dosage of 300 mg kg<sup>-1</sup> reduced body weight gain by 29% and decreased body fat accumulation by 50%, without altering food intake. Lifestyle modifications are the first-line therapy for patients with overweight or obese. Combined with physical activity (*e.g.*, swinging), TBs further enhanced weight control and insulin sensitivity in rats,<sup>65</sup> supporting its application as an adjunctive dietary strategy for individuals with overweight or obesity.<sup>66</sup>

As shown in Fig. 3, TBs regulate lipid metabolism and exert anti-obesity effects mainly through various mechanisms: (i) *Adipogenesis and lipogenesis*: by repressing the expression of the adipogenic markers of Cebpα and Pparγ, TBs inhibited adipocyte differentiation and the subsequent adipose maturation, which prevents fat accumulation in adipocyte, displaying the excellent properties in preventing obesity development.<sup>67</sup> (ii) *Thermogenesis*: in addition to inhibiting lipid synthesis, TBs prevent obesity development by promoting lipid burning or energy expenditure. TBs from Fuzhuan brick tea activated AMPK-PGC1α signaling, upregulated thermogenic gene expression such as *Ucp1*, *Prdm16*, and *Pgc1α*, and promoted energy expenditure, which reduced adipocyte expansion in HFD-fed mice.<sup>68</sup> (iii) *Fatty acid (FA) oxidation and lipolysis*: additionally, TBs accelerate lipid catabolism, exhibiting similar effects as black teas, which promote fatty acid oxidation and lipid utilization, thus preventing lipid accumulation and fat content in the body.<sup>69</sup> High molecular weight fractions of TBs (>50 kDa) from Pu-erh tea enhanced fatty acid oxidation and lipolysis by increasing carnitine and glycine levels, and upregulated *Cpt1* and *Cpt2* expression.<sup>70</sup> Tibetan tea extract rich in TBs also stimulated lipid catabolism, reduced adipocyte hyperplasia, and upregulated lipolytic gene of *Pnpla2*.<sup>71</sup> (iv) *Lipid digestion and absorption*: TBs and their 3–100 kDa fractions inhibited pancreatic lipase and cholesterol esterase, reduced cholesterol micellization, and decreased lipid accumulation in zebrafish model.<sup>23</sup> The 10–30 kDa fractions containing high content of phenols, lipids, saccharides, and proteins, were especially effective in reducing lipid content, promoted cholesterol conversion to BAs and fecal BAs excretion.<sup>28</sup> (v) *BA metabolism*: TBs modulated gut microbiota-



**Table 1** Health-promoting benefits of theabrownins and the underlying molecular mechanisms from *in vitro* and *in vivo* studies

Treatment	TBs source	Cell line or animal models	Effects and molecular mechanisms	PubMed ID
2300 mg per kg per BW for 14 weeks	TBs from dark tea	HFD-induced mice	Prevented NAFLD and obesity, improved FA oxidation, lipolysis, and oxidative stress <i>via</i> serotonin-related signaling.	36639024
300 mg per kg per BW for 7 weeks	TBs from dark tea	HFD-induced mice	Ameliorated obesity by modulating gut microbiota, reduced BW gain and fat accumulation, and promoted lipid clearance.	36230076
281.2–1125 mg per kg per BW for 9 weeks	TBs from Pu-erh tea	High-fat, sugar, and salt-fed rats	Improved serum lipid profiles, prevented obesity and IR, activated circadian rhythms, accelerated nutrient metabolism, and glucose-lipid depletion.	31273522
400 mg per kg per BW for 24 weeks	TBs from raw and ripened Pu-erh tea	HFD-induced mice	Improved gut microbiota disorders, inhibited BW gain, improved insulin sensitivity and glucose homeostasis, and reduced chronic inflammation.	37024732
200–800 mg per kg per BW for 12 weeks	TBs from Fuzhuan brick tea	HFD-induced mice	Improved gut homeostasis, reduced BW, improved dyslipidemia and glycaemia, and reduced inflammatory gene expression.	34581185
225 mg per kg per BW for 8 weeks	TBs from Pu-erh tea	HFD-induced mice	Shifted BA biosynthesis from classical to alternative pathway, improved obesity and energy metabolism by altering the gut microbiota.	33238385
225 mg per kg per BW for 8 weeks	TBs from Pu-erh tea	HFD-induced mice	Reduced cholesterol levels and blood lipids by regulating the gut microbiota and BA metabolism.	31672964
200–1000 $\mu\text{g mL}^{-1}$ for 48 hours	TBs from Pu-erh tea	Hyperlipidemia zebrafish	Improved lipid profiles by inhibiting intestinal lipid absorption and the enzymatic activities of pancreatic lipase and cholesterol esterase.	36252371
200–1000 $\mu\text{g mL}^{-1}$ for 48 hours	TBs from dark tea	Hyperlipidemia zebrafish	Decreased lipid levels in high-fat-induced zebrafish, with TBs (10–30 kDa) showing the highest effects.	32019226
1125 mg per kg per BW for 2 weeks	TBs from Pu-erh tea	HFD-induced rats	Prevented obesity, accelerated lipid metabolism, and increased carnitine levels by modulating serotonin-related signaling.	26676261
0.25% (w/v) for 10 weeks	Tibetan tea	HFD-induced mice	Promoted lipolysis, reduced obesity and white adipose tissue inflammation, reduced adipocyte proliferation and immune cell infiltration, and enhanced glutamine synthesis.	38047533
1124 mg per kg per BW for 63 days	TBs from Pu-erh tea	HFD-induced rats	Reduced BW and triglycerides levels, improved IR by targeting the reproduction of intestinal microorganisms.	31621728
400 and 800 mg per kg per BW for 6 weeks	TBs from Fuzhuan brick tea	HFD-induced hamsters	Ameliorated dyslipidemia, hepatic steatosis, and systemic inflammation, improved dysbiosis, and induced changes in microbiota-derived metabolites.	38567990
1.215 g per kg per BW for 16 weeks	TBs from Pu-erh tea	HFD-induced rats	Improved miRNA expression profile, alleviated metabolic syndrome, and restored intestinal flora balance.	39638201
180 and 360 mg per kg per BW for 8 weeks	TBs from Qingzhuan brick tea	HFD-induced mice	Prevented MASLD and improved dyslipidemia by increasing intestinal SCFAs, increasing Atgl, Ppar $\alpha$ , Ffar2, Ffar3 expression, and decreasing Lxr $\alpha$ , Srebp1c, Fas, and Hmger expression.	38642504
3 g per kg per BW for 12 weeks	—	HFD-induced mice	Improved lipid metabolism, prevented obesity by regulating BA and FA metabolism, and reduced serum and hepatic lipid content.	35833020
200 mg per kg per BW for 4 weeks	TBs from Fuzhuan brick tea	MCD-induced mice	Ameliorated liver injury, inflammatory response, oxidative stress, and fibrosis by increasing Fgf21 levels and decreasing p38-MAPK signaling.	36742397
100–400 mg per kg per BW for 8 weeks	TBs from Fuzhuan brick tea	HFD-induced mice	Reduced obesity, improved IR, liver inflammation, and macrophage infiltration by altering gut microbial composition and metabolites.	38563324
150 $\mu\text{g mL}^{-1}$ for 24 hours	TBs from dark tea	Insulin resistant-HepG2 cells	Ameliorated oxidative stress, improved glycogen synthesis and glucose consumption, inhibited gluconeogenesis and FA synthesis.	37764646
200–400 mg per kg per BW for 4 weeks	TBs from Pu-erh tea	T2DM-induced mice	Delayed glucose absorption, promoted tryptophan metabolism, regulated LPS/GLP-1 levels, and restored pancreatic beta cell function.	40397806
2300 mg per kg per BW for 10 weeks	TBs from Pu-erh tea	GDM offspring mice	Improved skeletal muscle function, reduced lipid accumulation, regulated BCAA metabolism and fatty acid biosynthesis pathways.	40327367
625 mg per kg per BW for 8 weeks	TBs from Pu-erh tea	Goto-Kakizaki rats	Improved diabetes and IR by modulating gut microbiota, reduced blood glucose levels, and lipid metabolism.	35088787
62.5–1500 $\mu\text{g mL}^{-1}$ for 24 or 48 hours	TBs from dark tea	LPS-induced RAW264.7 cells	Inhibited LPS-induced inflammation and enhanced innate immunity through TLR2/4 signaling.	37048289



Table 1 (Contd.)

Treatment	TBs source	Cell line or animal models	Effects and molecular mechanisms	PubMed ID
50–400 $\mu\text{g mL}^{-1}$ for 24 hours & 100–1000 mg per kg per BW for 21 days	TBs from Tibetan tea	LPS-induced RAW264.7 cells & LPS-induced in mice	Reduced LPS-induced inflammation <i>via</i> NF- $\kappa$ B/Nrf2 pathway, altered M1/M2 macrophage ratio, regulated purine and amino acid metabolism.	40245629
1215 mg per kg per BW for 7 or 26 days	TBs from Pu-erh tea	DSS-induced mice	Ameliorates ulcerative colitis by restoring intestinal homeostasis with increased occludin, Claudin-1, and Muc2 expression, inhibiting the release of pro-inflammatory cytokines.	39029137
180 and 360 mg per kg per BW for 8 weeks	TBs from Qingzhuan brick tea	HFD-induced mice	Inhibited neuroinflammation, synaptic plasticity, and neuronal apoptosis, protected hippocampal injury by modulating MARK4/NLRP3 signaling.	38455533
215 mg per kg per BW for 12 weeks	TBs from Pu-erh tea	Metabolic syndrome mice	Improved glycerophospholipid metabolism and linoleic acid metabolism by modulating the gut microbiota and lipid metabolism.	36461373
0.25% (w/v) for 60 days	Ripened Pu-erh tea	Circadian rhythm disorder in mice	Improved gut microbiota disorder, inhibited the infiltration of harmful microorganisms or metabolites through enterohepatic circulation, and reduced chronic liver inflammation.	34726418
0.25% (w/v) for 70 days	Pu-erh tea	Circadian rhythm disorder in mice	Inhibited obesity through the metabolite-blood circulation-adipose tissue axis, promoted lipolysis and thermogenesis, improved lipid accumulation, and accelerated glycolipid metabolism.	35749873
1.215 g per kg per BW for 16 weeks	TBs from Pu-erh tea	HFD-induced mice	Improved dyslipidaemia, remodeled gut microbiota circadian rhythm, regulated bile acid metabolism, and reduced obesity in mice.	39866149
50–200 $\mu\text{g mL}^{-1}$ for 24 hours	TBs from Tibetan tea	Ethanol-induced zebrafish	Improved ethanol-induced defects, regulated phosphatidylethanolamine and amino acid metabolism, alleviated oxidative stress, and maintained cellular homeostasis.	39967082
28–280 mg per kg per BW for 4 weeks	TBs from black tea	HUA-induced mice	Reduced serum uric acid by inhibiting XOD, upregulated OAT1/OAT3 transporters, alleviated renal fibrosis <i>via</i> PI3K/AKT pathway.	39743074
40–400 mg per kg per BW for 24 hours	TBs from black tea	AKI-induced mice	Increased levels of GAA and FA in the kidneys, reduced renal cell apoptosis and ferroptosis, and improved kidney damage.	40090045
75–675 mg per kg per BW for 14 days	TBs from Tibetan tea	UVB-induced mice	Prevented UVB-induced photodamage, improved the body's antioxidant capacity, reduced skin inflammation, prevented collagen destruction, and inhibited cell apoptosis.	39638201

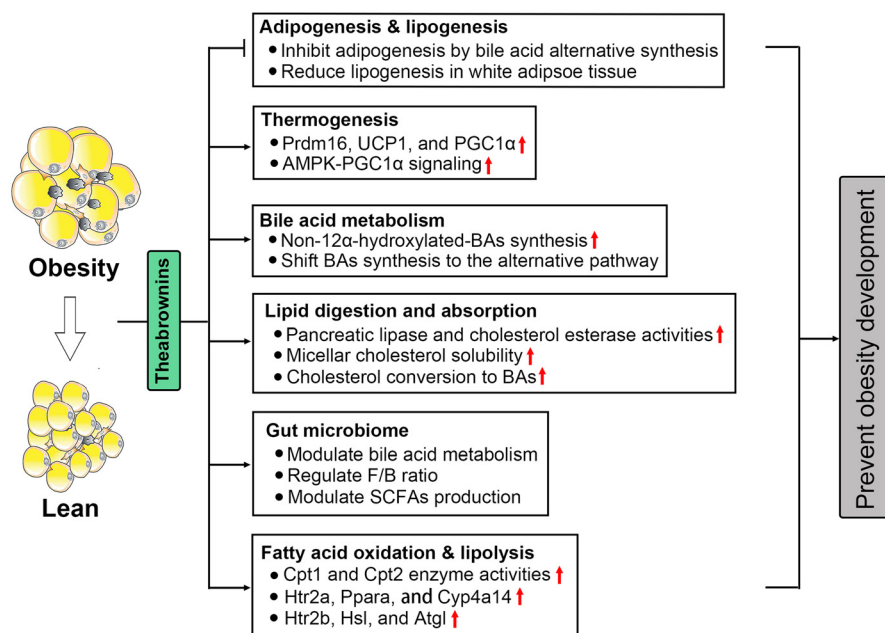
BW, body weight. “—”, not mentioned.

mediated BA biosynthesis by shifting from the classical to the alternative pathway, leading to increased non-12 $\alpha$ -hydroxylated BAs levels and energy-metabolism related gene expression in adipose tissues, including Ucp1, Dio2 and Pgc1 $\alpha$ , which attenuated obesity in mice and revealed a novel microbiota-BA-adipose tissue axis for metabolic regulation.<sup>72,73</sup> (vi) *Gut microbiome*: TBs undergo biotransformation in the gastrointestinal tract and influence microbial composition (Fig. 3). The resulting metabolites further regulate lipid metabolism, a topic explored in greater detail below (section 4.1.2).

Obesity-related dyslipidemia is marked by elevated triglycerides, very low-density lipoprotein (VLDL), and apolipoprotein B (ApoB), along with reduced high-density lipoprotein cholesterol (HDL-C) and increased low-density lipoprotein cholesterol (LDL-C) levels.<sup>74,75</sup> TBs from Pu-erh tea significantly reduced hepatic and serum triglyceride contents, modulated cholesterol levels, and ameliorated lipid metabolism in HFD-fed mice and human participants.<sup>73</sup> In cell-based models, TBs lowered triglycerides and cholesterol levels in oleic acid-treated HepG2 cells, highlighting their hepatoprotective potential.<sup>76</sup>

High-sugar diets promote *de novo* lipogenesis and VLDL overproduction, exacerbating dyslipidemia.<sup>77</sup> TBs intervention reversed these effects in high-sugar diet-fed rats, reducing body weight, triglyceride levels, and improving insulin resistance (IR).<sup>78</sup> Moreover, TBs supplementation in hamsters improved serum lipid profiles and attenuated metabolic disorders induced by HFD, reinforcing their roles in dyslipidemia management.<sup>15</sup> Recent studies have found that TBs may alleviate skeletal muscle dysfunction in the offspring of a mouse model of gestational diabetes mellitus by modulating the biosynthesis of unsaturated fatty acids.<sup>79</sup> Collectively, TBs offer a promising, safe, and cost-effective intervention for obesity and associated metabolic disorders. Their multi-target actions, ranging from thermogenesis to BA metabolism and gut microbiota modulation, position them as potent candidates for functional food applications aimed at weight control and dyslipidemia prevention. As a dietary strategy, TBs could be incorporated into health regimens for individuals with overweight, obesity, or metabolic syndrome, providing therapeutic benefits with minimal adverse effects.





**Fig. 3** Proposed mechanisms underlying the anti-obesity effects of TBs include the activation of thermogenesis, inhibition of adipogenesis and lipogenesis, enhancement of fatty acid oxidation and lipolysis, promotion of lipid digestion and absorption, and modulation of bile acid metabolism and gut microbiota composition.

**4.1.2 Anti-fatty liver disease.** Non-alcoholic fatty liver disease (NAFLD), one of the most common chronic liver conditions, is clinically categorized based on disease progression into simple steatosis, non-alcoholic steatohepatitis (NASH), and NASH-related cirrhosis, depending on the extent of hepatic lipid accumulation, inflammation, and fibrosis.<sup>80</sup> A recent meta-analysis reported that the global prevalence of NAFLD rose from 25.3% (1990–2006) to 38.0% (2016–2019), emphasizing the urgent need for safe and effective interventions.<sup>81</sup> Owing to their potent lipid-regulating and anti-inflammatory properties, TBs have shown promise in ameliorating NAFLD by preventing hepatic lipid accumulation and impeding the transition from steatosis to steatohepatitis. (i) *Liver steatosis*: several studies have demonstrated that TBs inhibited liver fat accumulation and protected against diet-induced hepatic steatosis. In HFD-induced mouse models, TBs from Qingzhuan brick tea (180 and 360 mg per kg per day, 8 weeks) significantly reduced liver weight, hepatic alanine transaminase (ALT) and aspartate aminotransferase (AST) levels, and hepatic lipid accumulation, thereby preventing liver steatosis.<sup>53</sup> In HFD-fed hamsters, TBs supplementation (400 and 800 mg kg<sup>-1</sup>) significantly reduced hepatic triglycerides and free fatty acid levels, lowered serum ALT and AST contents, and decreased the atherosclerosis index, supporting their benefits for liver lipid homeostasis. Mechanistically, TBs suppressed the expression of key lipogenic genes, including *Srebp1c*, *Fas*, *Acc1*, and *Scd1*, downregulated *Lxr*, and upregulated *Fxr* expression, suggesting that TBs attenuate hepatic steatosis *via* modulation of the SREBP signaling pathway.<sup>10</sup> Positive hepatic lipid accumulation is due to the increased

hepatic lipogenesis and decreased lipid oxidation and lipid export from liver cells, regulated by SREBP, PPAR, AMPK, and PKA signaling pathways.<sup>82</sup> Activation of PPAR signaling, induction by the agonists of fenofibrate or pemafibrate, robustly induced fatty acid oxidation and reduced hepatic lipid accumulation.<sup>83</sup> However, due to the side effects of inducing hepatomegaly and potent tumorigenesis in the livers of rodent animals, fenofibrate is limited in patients with hypertriglyceridemia.<sup>84</sup> TBs are natural products from fermented dark teas or black teas, which have shown efficacy in preclinical models. Similarly, TBs lowered total hepatic ceramide levels and suppressed the farnesoid X receptor (FXR) signaling pathway, which is implicated in lipid metabolism and ceramide biosynthesis. TBs may reduce body weight gain and body fat rate, decrease hepatic lipid accumulation, and inhibit NAFLD in mice, *via* improvement of fatty acid oxidation, lipolysis, and oxidative stress. The untargeted metabolomics and ELISA assay demonstrated that TBs decreased serotonin levels in the liver and increased the levels in blood circulation and visceral white adipose tissue, suggesting a complicated mechanism and the different effects on lipid metabolism in different organ sites by regulating serotonin-related signaling pathways.<sup>63,85</sup> Additionally, a combination of TBs (0.3% w/w) and Poria mushroom (*Poria cocos*) polysaccharide (0.15% w/w) administered for 8 weeks significantly reduced hepatic triglycerides and cholesterol levels in obese mice.<sup>86</sup> (ii) *NASH*: this condition represents a more advanced and inflammatory stage of NAFLD. In a methionine- and choline-deficient diet (MCD) induced mouse model, TBs administration (200 mg per kg per day for 4 weeks) alleviated hepatic steatosis, prevented liver



mass loss, and improved histopathological liver injury by inducing *Fgf21* expression and suppressing the AMPK/p38 signaling pathway, thereby reducing hepatic inflammation and halting NASH progression.<sup>87</sup> FGF21 can modulate the “multi-hits” in the pathogenesis of fatty liver disease, is secreted primarily from the liver cells, decreases *de novo* lipogenesis, and activates fatty acid oxidation, which prevents the development of fatty liver disease, illustrating the lipid-lowering roles *via* the *Fgf21*-dependent pathway.<sup>88</sup> Furthermore, TBs reduced hepatic expression of pro-inflammatory genes such as *Mcp-1*, *Tnf- $\alpha$* , *Il-1 $\beta$* , and *Il-6* in a high-fat/high-cholesterol diet-induced model, supporting their anti-inflammatory effects in the liver.<sup>15</sup> Together, these findings underscore the multifaceted role of TBs in preventing and managing fatty liver disease.

**4.1.3 Anti-diabetes.** Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from insufficient insulin production or impaired insulin utilization.<sup>89</sup> Hyperglycemia leads to elevated blood glucose levels and is a major contributor to cardiovascular disease, renal impairment, and neuropathy. TBs from Fuzhuan brick tea significantly reduced blood glucose levels at multiple time points (30, 60, 90, and 120 minutes) during oral glucose tolerance tests (OGTT) and reduced AUC (area under the curve) values, reflecting improved glycemic control.<sup>12</sup> Enhanced glucose control is always achieved through increased insulin sensitivity. Evidence suggests that TBs, especially when combined with plant-derived polysaccharides (*e.g.*, Poria mushroom polysaccharides), can significantly improve glycemic control by enhancing insulin sensitivity and modulating glucose metabolism.<sup>86</sup> Ninghong black tea TBs improved insulin sensitivity, reduced hepatic gluconeogenesis and glucose production, and increased glucose consumption by reducing FoxO1-PGC1 $\alpha$  activities and activating AMPK signaling, suggesting the protective roles against diabetes.<sup>67</sup> IR is a hallmark of T2DM.<sup>90</sup> TBs reduced the homeostatic model assessment of insulin resistance (HOMA-IR) index in HFD-fed diabetic mice, suggesting improvements in insulin sensitivity. Moreover, TBs exhibit robust anti-diabetes bioactivities in diabetic models. In a db/db mouse model of T2DM, TBs delayed glucose absorption by inhibiting  $\alpha$ -glucosidase in the intestine, regulated LPS/GLP-1 levels, and improved  $\beta$ -cell dysfunction.<sup>91</sup> In Goto-Kakizaki rats, a well-established non-obese T2DM model characterized by moderate hyperglycemia, impaired pancreatic  $\beta$ -cell function, and chronic inflammation, TBs from Pu-erh tea significantly decreased fasting blood glucose and HOMA-IR index;<sup>12</sup> and upregulated the amounts of beneficial metabolic hormones and enzymes, including adiponectin, leptin, and glucokinase, *via* modulation of the gut microbiota.<sup>92</sup> Mechanistic studies have shown that persistent hyperglycemia induces oxidative stress and contributes to IR by impairing the intracellular insulin signaling pathway.<sup>93</sup> In insulin-resistant HepG2 cells, TBs enhanced glucose metabolism by restoring IRS-1/PI3K/Akt signaling, reducing reactive oxygen species (ROS) levels, and improving cellular insulin response.<sup>8,91</sup> Epidemiological studies further support the roles of TBs and dark tea consumption in diabetes

prevention. A population-based survey of 1923 participants found that regular intake of dark tea was associated with a lower risk of dysglycemia and elevated urinary glucose, supporting its preventive role in T2DM management.<sup>94</sup>

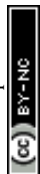
## 4.2 Antioxidant

A physiological balance normally exists between ROS production and antioxidant defenses. Oxidative stress resulting from an imbalance leads to DNA damage, cell death, and necrosis, and is implicated in the pathogenesis of cancer and T2DM.<sup>95,96</sup> The antioxidant capacity of TBs is greatly determined by their chemical composition. Rich in phenolic hydroxyl groups (*e.g.*, catechol and resorcinol), TBs effectively scavenge free radicals *via* hydrogen atom transfer or electron donation mechanisms.<sup>97–99</sup> Low molecular weight fractions of TBs (<10 kDa) exhibit stronger radical scavenging due to greater exposure of active sites, while high molecular weight fractions tend to chelate metal ions, which indirectly inhibit oxidative reactions.<sup>47,100</sup>

Published studies have outlined the antioxidant mechanisms of TBs: (i) *direct scavenging of reactive oxygen radicals*: *in vitro* models showed that TBs exerted stronger antioxidant activity by eliminating hydroxyl radicals compared to vitamin C (ascorbic acid).<sup>101,102</sup> (ii) *Enhancing the activity of antioxidant enzymes*: TBs from Tibetan tea increased the mRNA and protein levels of Nrf2, enhanced SOD enzyme activity, and improved the antioxidant capacity, which prevented UVB-induced skin photodamage by inhibiting MAPK/NF- $\kappa$ B signaling pathway and activating Nrf2 signaling pathway, offering the future applications in skin health promotion.<sup>103</sup> TBs activate the Nrf2/HO-1 signaling pathway, increased the expression of HO-1 and GST genes, and upregulated hepatic catalase (CAT) and superoxide dismutase (SOD).<sup>101,104</sup> (iii) *Inhibiting oxidase activity*: TBs inhibited xanthine oxidase (XO) and lipoxigenase (LOX), reducing ROS formation. They also inhibited  $\alpha$ -glucosidase and pancreatic lipase ( $IC_{50}$  = 0.32 and 0.45 mg mL<sup>-1</sup>, respectively), indirectly lowering oxidative stress risk.<sup>47,97–99</sup>

## 4.3 Anti-inflammation

Inflammation plays a central role in the development and progression of a range of chronic diseases, including cardiovascular disease, inflammatory bowel disease, diabetes, arthritis, neurological disorders, and cancer.<sup>105,106</sup> In response to cellular injury, the body initiates an inflammatory reaction involving the release of pro-inflammatory cytokines, chemokines, and other signaling molecules aimed at removing damaged cells and eliminating the source of injury.<sup>107</sup> However, uncontrolled or persistent inflammation can lead to chronic tissue damage and further contribute to chronic diseases.<sup>108–110</sup> TBs have shown significant anti-inflammatory potential through multiple mechanisms, suggesting the protective roles in alleviating inflammation-associated diseases (Fig. 4). These mechanisms are demonstrated in reported studies, including (i) *systemic inflammation*: TBs from Pu-erh tea have been shown to reduce LPS-induced nitric oxide (NO)



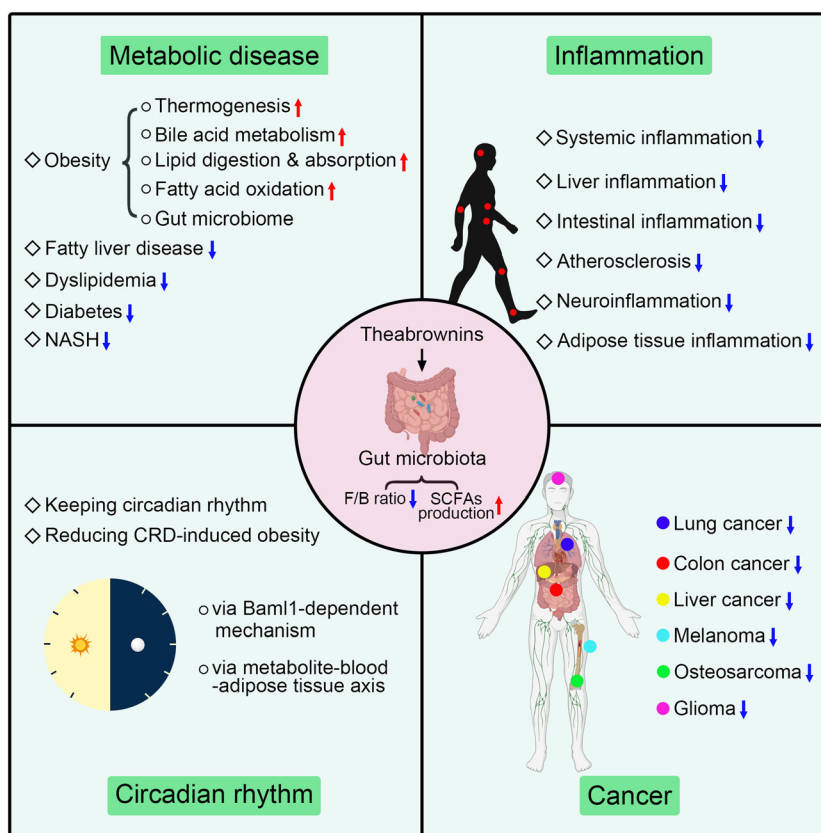
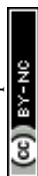


Fig. 4 Health-promoting effects of TBs, including alleviation of metabolic disorders, anti-inflammatory activity, regulation of circadian rhythms, and anti-cancer potentials.

secretion and cytokine production in RAW264.7 macrophages by attenuating MAPK and NF- $\kappa$ B signaling pathways while activating the PI3K/Akt signaling, thereby enhancing the innate immune response.<sup>111</sup> Similarly, TBs from Tibetan tea alleviated systemic inflammation by suppressing inflammatory gene expression and oxidative stress, and by decreasing the M1/M2 macrophage ratio in LPS-induced RAW264.7 cells through modulation of the NF- $\kappa$ B/Nrf2 signaling pathway.<sup>112</sup> However, future studies on the anti-inflammatory properties of TBs should proceed with caution. To date, most investigations have been limited to *in vitro* models assessing antioxidant, anti-inflammatory, and anti-cancer. Due to the low bio-availability of polyphenols and their limited interactions with cellular enzymes and genes, the findings from *in vitro* studies are often not reproducible *in vivo*. Therefore, it is highly recommended to adopt a “first *in vivo*, then *in vitro*” research strategy, or at a minimum, incorporate multiple cell lines to improve the reliability and translational value of the results.<sup>113</sup> (ii) *Liver inflammation*: in mice fed an MCD-diet, which induces NASH, TBs treatment significantly reduced hepatic inflammation and liver injury. This was evidenced by decreased F4/80, Ly-6 g, and IL-1 $\beta$  staining in the liver, along with reduced expression of *Mcp-1* and *Icam-1* genes, *via* down-regulation of MAPK/ERK and NF- $\kappa$ B signaling pathways.<sup>87</sup> (iii) *Adipose tissue inflammation*: TBs from Fuzhuan brick tea

lowered the expression of inflammatory genes of *Mcp1*, *Il-6*, and *Tnf- $\alpha$*  in white and brown adipose tissues from HFD-fed mice, thereby reducing systemic adipose inflammation.<sup>68</sup> (iv) *Neuroinflammation*: in mice subjected to long-term HFD-feeding, TBs from Qingzhuang brick tea mitigated hippocampal injury by reducing astrocyte and microglia activation, downregulating *MARK4* and *NLRP3* expression, and preventing neuronal apoptosis. These effects were accompanied by decreased *Bax* and increased *Bcl-2* expression, suggesting modulation of the MARK4/NLRP3 signaling axis.<sup>114</sup> (v) *Intestinal inflammation*: in a dextran sulfate sodium (DSS)-induced colitis mouse model, TBs from Pu-erh tea suppressed pro-inflammatory cytokines release, inhibited Th1 and Th17 cell differentiation in the colonic lamina propria, and promoted a shift in the Treg/Th17 balance toward Tregs, which was linked to the inhibition of TLR2/4 signaling pathway.<sup>14</sup> (vi) *Atherosclerosis*: a study compared the anti-inflammatory and anti-atherosclerotic effects of teas before and after microbial fermentation. *Monascus purpureus*-fermented Pu-erh tea extract more effectively reduced the contents of serum triglycerides, total cholesterol, LDL-C, and inflammatory cytokines of *Tnf- $\alpha$* , *Il-1 $\beta$* , and *Il-6*, compared to sun-dried green tea. The fermented tea also prolonged thrombosis time, reduced foam cell formation, and protected endothelial cells, suggesting more potent anti-inflammatory and anti-atherogenic properties.<sup>49</sup>



The anti-inflammatory effects of TBs are attributed in part to their rich content of flavonoids and phenolic compounds,<sup>115</sup> which also exert anti-oxidant activities.<sup>116</sup> This topic is described in more detail in section 4.6.

#### 4.4 Antiproliferative and anti-tumor

In 2022, nearly 20 million new cancer cases and 9.7 million cancer-related deaths were reported globally. Among the cases, lung cancer was the most commonly diagnosed (12%), followed by breast (12%), colorectal (10%), prostate (7%), and stomach cancer (5%).<sup>117</sup> Cancer treatment is complex, and natural products have long contributed to anticancer drug discovery.<sup>73,118,119</sup> TBs, as dietary bioactives, have been studied for anti-tumor potential in various models across various cancer types. Their underlying molecular mechanisms are summarized as follows (Table 2 and Fig. 4). (i) *Lung cancer*: TBs inhibited the proliferation of A549 cells by inducing G0/G1 cell cycle arrest, downregulating proliferation markers including *c-Myc*, *Cyclin A*, *Cyclin D*, *Cdk2*, *Cdk4*, and *Pcna*, while upregulating *p21*, *p27*, and *Pten* expression. Furthermore, TBs treatment reduced Lewis lung carcinoma tumor incidence and tumor volume, exhibiting inhibiting effects on lung cancer growth in mice.<sup>120</sup> TBs also inhibited the proliferation of non-small cell lung adenocarcinoma cells, downregulated *Topo I*, *Topo II*, and *Bcl-2* gene expression, and upregulated pro-apoptotic genes including *E2f1*, *p53*, *Gadd45*, *Bax*, and *Bim*, activating p53-mediated apoptosis.<sup>121</sup> Additional studies using cell lines such as A549, H2030, HCC827, H1975, and PC9 supported TBs antiproliferative effects. TBs inhibited proliferation, promoted apoptosis, and induced G1 phase

arrest through suppression of the PI3K/Akt/mTOR signaling pathway in cancer cells.<sup>122</sup> (ii) *Colon cancer*: TBs derived from enzymatically and chemically oxidized tea polyphenols inhibited growth and induced late-stage apoptosis in colon cancer cells.<sup>11</sup> In an azoxymethane (AOM)- and DSS-induced mouse model of colorectal cancer, TBs reduced tumor count, improved crypt structure, and decreased colon fibrosis. TBs also downregulated Ki67 expression, exerting anti-tumor effects *via* repression of PI3K/Akt/mTOR signaling.<sup>123,124</sup> (iii) *Liver cancer*: green tea-derived TBs induced cellular senescence and apoptosis in hepatocellular carcinoma (HCC) cells by activating the p53 signaling pathway and inhibiting the JNK signaling pathway. In zebrafish xenograft models, TBs suppressed tumor growth through anti-angiogenic and immunomodulatory effects.<sup>125</sup> Additionally, TBs reduced Huh7 cell proliferation and promoted apoptosis by upregulating *Noxa*, *Puma*, *p21*, *Bax*, and *Bim* expression *via* the JNK signaling to mediate tumor suppression.<sup>126</sup> (iv) *Melanoma*: in melanoma A375 cells, TBs inhibited proliferation, induced DNA damage, and triggered apoptosis. In zebrafish xenograft models, TBs suppressed tumor growth through mechanisms involving the p53/NF- $\kappa$ B signaling axis.<sup>127</sup> (v) *Osteosarcoma*: metastasis is a major contributor to cancer mortality.<sup>128</sup> TBs inhibited migration and invasion of U2OS osteosarcoma cells and suppressed epithelial-mesenchymal transition (EMT) by downregulating *Vimentin*, *Slug*, *Snail-1*, and  $\beta$ -*Catenin* expression, and upregulating *Claudin-1* and *E-cadherin* expression *via* NF- $\kappa$ B signaling inhibition.<sup>129</sup> Furthermore, TBs induced DNA damage and apoptosis, regulating *Mki67*, *PARP*, and *Caspase-3* expression through a p53-dependent pathway.<sup>130</sup> (vi) *Glioma*: in glioma

**Table 2** Anti-cancer bioactivities of TBs from *in vitro* and *in vivo* studies

Cancer type	Cell line or animal models	Regulated genes and effects	Related mechanisms	PubMed ID
<b>Lung cancer</b>	A549 cells and zebrafish	Increase <i>c-Myc</i> , <i>Cyclin A</i> , <i>Cyclin D</i> , <i>Cdk2</i> , <i>Cdk4</i> , and <i>Pcna</i> expression, and decrease <i>p21</i> , <i>p27</i> , and <i>Pten</i> expression; decrease tumor incidence and tumor volume.	—	28289384
	A549 cells	Decreased <i>TopoI</i> , <i>TopoII</i> , and <i>Bcl-2</i> expression, and increased <i>E2F1</i> , <i>p53</i> , <i>Gadd45</i> , <i>Bam</i> , and <i>Bim</i> expression.	p53 signaling	27994550
	A549, H2030, HCC827, H1975, PC9 cells and nude mice	Reduced mTOR and <i>Cyclin D1</i> expression led to G1 arrest; inhibited cell proliferation; induced apoptosis and autophagy; and reduced A549 xenograft tumor growth	PI3K/Akt signaling	35529455
<b>Colon cancer</b>	HT-29 cells	Inhibited cell growth and caused late apoptosis.	—	35369086
	AOM/DSS-treated mice	Reduced tumor count, improved crypt length and colon fibrosis, reduced Ki67 expression, and tumorigenesis.	PI3K/Akt/mTOR signaling	36139789
<b>Liver cancer</b>	SK-Hep-1, HepG2, Huh7 cells, and zebrafish	Induced cellular senescence and apoptosis; inhibited HCC tumor growth.	p53 and JNK signaling	35078476
	Huh7 cells and zebrafish	Inhibited cell proliferation, induced apoptosis by inducing <i>Noxa</i> , <i>Puma</i> , <i>p21</i> , <i>Bax</i> , and <i>Bim</i> expression, and inhibited HCC tumor growth.	JNK signaling	32982289
<b>Melanoma</b>	A375 cells	Inhibited cell proliferation, induced DNA damage, and apoptosis.	p53 and NF- $\kappa$ B signaling	35779042
<b>Osteosarcoma</b>	U2OS cells	Prevented EMT, decreased <i>Vimentin</i> , <i>Slug</i> , <i>Snail-1</i> , and $\beta$ - <i>catenin</i> expression, and increased <i>Claudin-1</i> and <i>E-cadherin</i> expression.	NF- $\kappa$ B signaling	33125106
	U2OS cells	Induced DNA damage and apoptosis; regulated <i>Mki67</i> , <i>PARP</i> , and <i>Caspase3</i> expression.	p53 signaling	29993186
<b>Glioma</b>	A172, U87, U251, and HOG cells	Induced cell growth arrest, decreased <i>c-Myc</i> , <i>Cyclin D</i> , <i>Cdk2</i> , and <i>Cdk4</i> expression, and increased <i>p21</i> and <i>p27</i> expression.	p53 signaling	33995088

“—”, not mentioned.





circadian desynchrony in HepG2 cells by regulating both mRNA and protein levels of circadian genes. These findings suggest that TBs exert protective metabolic effects *via* a Bmal1-dependent mechanism.<sup>76</sup> In modern society, lifestyle factors such as business travel, night shifts, and late-night social activities have become common contributors to circadian rhythm disorders (CRD). CRD induced by chronic light disruption led to increased body weight gain, elevated blood glucose, enhanced hepatic lipid accumulation, dyslipidemia, and IR. Ripened Pu-erh tea effectively mitigated these outcomes by enhancing BA-mediated enterohepatic circulation, indicating its therapeutic potential in managing CRD.<sup>134</sup> Another study reported that Pu-erh tea promoted the expression of lipolysis- and thermogenesis-related genes in white adipose tissue, including *Hsl*, *Atgl*, *Ppara*, *Ckb*, and *Ucp1*, which reduced CRD-induced obesity *via* modulating the metabolite-blood circulation-adipose tissue axis.<sup>135</sup> Exercise is another effective intervention for maintaining circadian rhythm and resetting the molecular clock.<sup>136</sup> Interestingly, a study showed that combining TBs intake with physical activity (*i.e.*, swinging) synergistically modulated hepatic circadian gene expression by downregulating *Bmal1* and *Clock* and upregulating *Per2* and *Per3* genes. These findings support that TBs may ameliorate obesity and IR in a metabolic syndrome rat model.<sup>65</sup> Although these studies supported the regulatory potential of TBs on circadian rhythms, the underlying molecular mechanisms remain largely undefined. Recent studies suggest that the potential of TBs to regulate circadian rhythms is likely to be based on their remodeling of the rhythmic oscillations of gut microbes,<sup>137</sup> which are achieved through L-palmitoylcarnitine regulation of *Per3* expression in diabetic KKAY mice.<sup>138</sup>

#### 4.6 Improvement of gut microbiota dysbiosis

The gut microbiota is increasingly recognized as a virtual organ due to its profound impact on host biology and physiology. A growing body of evidence suggests that gut microorganisms play a key role in regulating metabolic health and are closely associated with the pathogenesis of obesity, T2DM, NAFLD, cardiometabolic disorders, and malnutrition.<sup>139</sup> Microbes residing in the gastrointestinal (GI) tract metabolize dietary components into bioactive metabolites, serving as a critical interface between the diet and host metabolism. Ingested TBs undergo microbial fermentation in the GI tract, producing smaller bioactive and more bioavailable compounds that may be absorbed into the bloodstream and influence metabolic pathways. These metabolites may also interact with BA metabolism and lipid regulation. However, the specific metabolic transformations of TBs in the gut and their downstream bioactivities remain poorly characterized. Conversely, TBs can also modulate gut microbial composition, thereby exhibiting mutual regulatory effects.<sup>140</sup> The effects of TBs on gut microbiota are summarized in the following aspects.

(i) Modulation of BA metabolism: TBs from Pu-erh tea have been shown to reshape gut microbial communities in both mice and humans, increasing the abundance of the beneficial genera such as *Lactobacillus*, *Bacillus*, *Streptococcus*, and

*Lactococcus*, while suppressing microbes associated with bile salt hydrolase activity. This modulation can lead to elevated ileal conjugated BA levels, enhanced hepatic BA synthesis, and increased fecal BA excretion, collectively lowering hepatic cholesterol and lipogenesis in the liver.<sup>73</sup> In addition, TBs increased the abundance of *Clostridium scindens* and *Parabacteroides distasonis*, promoting secondary BAs production, which improved lipid metabolism in adipose tissues.<sup>72</sup>

(ii) Modulation of *Firmicutes* to *Bacteroidetes* (F/B) ratio: an elevated F/B ratio has been linked to obesity and systemic inflammation.<sup>141</sup> TBs have been reported to restore gut microbiota homeostasis in HFD-induced dysbiosis. Specifically, TBs from Pu-erh tea reduced the F/B ratio by increasing the abundance of *Bacteroidetes* such as *Prevotella* sp. CAG:1031, *Prevotella* sp. MGM2, and *Bacteroides sartorii*, while reducing *Firmicutes* species like *Roseburia* sp. 1XD42-69 and *Roseburia* sp. 831b. These alternations improved serum lipid profiles, reduced fasting blood glucose and hemoglobin A1c levels, improved glycerophospholipid metabolism, arachidonic acid metabolism, and glycolysis/gluconeogenesis, further reduced IR.<sup>142</sup> TBs from Fuzhuan brick tea decreased the abundance of pro-inflammatory *Firmicutes* genera, including *Colidextribacter*, *Acetatifactor*, *Blautia*, *Lactobacillus*, and *Romboutsia*, while increasing beneficial *Verrucomicrobiota* such as *Akkermansia*. This microbial shift elevated bioactive metabolites like L-ornithine,  $\alpha$ -ketoglutarate, and glutamine, ultimately reducing adiposity and systemic IR in obese mice.<sup>12</sup> Moreover, TBs reduced the abundance of *Ruminococcus*, which is a genus associated with lipid metabolism disorders in NAFLD,<sup>143</sup> while increasing the abundance of the health-promoting genera including *Blautia*, *Tuzzerella*, *Odoribacter*, *Butyricimonas*, *Lachnoclostridium*, *Bilophila*, and *Alistipes*.<sup>15</sup> Notably, *Alistipes* has been inversely correlated with obesity and dyslipidemia, and its level was reduced in women with gestational diabetes.<sup>144</sup> *Butyricimonas virosa* supplementation in HFD-fed mice improved diabetic parameters, including body weight, blood glucose, insulin sensitivity, and liver steatosis through GLP-1 receptor activation.<sup>145</sup> Notably, raw and ripened Pu-erh teas effectively suppressed weight gain, enhanced insulin sensitivity, and improved glucose homeostasis in HFD-fed mice. TBs interventions increased microbial diversity and richness, as reflected in elevated ACE, Chao1, and Shannon index, while reducing the Simpson index. The F/B ratio in the raw Pu-erh tea group was significantly lower than in the ripened tea group and closely resembled that of non-obese mice, suggesting a likely superior effect of raw Pu-erh tea in reversing dysbiosis by promoting the abundance of *Lactobacillus* and *Lachnospiraceae\_NK4A136\_group*.<sup>64</sup>

(iii) Modulation of short-chain fatty acids (SCFAs) production: SCFAs (*i.e.*, acetate, propionate, and butyrate) are beneficial microbial metabolites produced through the fermentation of indigestible fibers and resistant starches.<sup>146</sup> Their levels are influenced by diet and environmental factors, such as antibiotic use, which in turn shape gut microbiota composition and function.<sup>147</sup> TBs from Qingzhuang brick tea



increased acetate, propionate, and butyrate concentrations in the intestines, which led to reduced lipid accumulation in the liver and adipose tissues in HFD-fed mice.<sup>53</sup> TBs also increased the abundance of SCFAs production-related genera, such as *Bacteroides*, *Blautia*, and *Lachnospirillum*.<sup>148,149</sup> Moreover, TBs supplementation (800 mg kg<sup>-1</sup>) reversed HFD-induced reductions in total SCFAs, particularly increasing intestinal butyrate.<sup>68</sup> Notably, TBs and Pu-erh tea reduced both obesity-related SCFAs (e.g., acetic and propionic acid) and harmful SCFAs (e.g., isobutyric and isovaleric acid), further supporting their roles in mitigating metabolic syndrome.<sup>122</sup>

#### 4.7 Other biological effects

Beyond the aforementioned biological activities, TBs exhibit additional physiological effects. TBs have been shown to inhibit RANKL-induced osteoclastogenesis by downregulating key osteoclast markers including *Nfatc1*, *Trap*, *c-Fos*, and *Cathepsin K* genes. In ovariectomized rats, TBs improved femoral bone mineral density, enhanced biomechanical strength, and ameliorated bone microarchitecture by increasing cortical thickness and trabecular bone area.<sup>150</sup> In poultry studies, TBs from Pu-erh tea improved ovarian function and enhanced production performance in laying hens, increasing egg weight and quality. These effects may be attributed to the activation of the SIRT1 signaling pathway.<sup>151</sup> TBs reduced serum uric acid levels and ameliorated renal pathological damage in hyperuricemic mice, and inhibited autophagy, inflammation, and fibrosis in the kidneys of hyperuricemia (HUA) mice, suggesting the potential application of TBs in HUA treatment.<sup>13</sup> They also reduced iron and malondialdehyde levels, increased glutathione content, along with increased *Gpx4* expression, which alleviated burn-induced ferroptosis; and ameliorated renal apoptosis and renal injury by increasing the levels of guanidinoacetic acid and fumaric acid in the kidneys.<sup>152</sup>

In summary, TBs function as natural antioxidants through multiple pathways, including free radical scavenging, enzyme modulation, and gut microbiome regulation. These properties support TBs applications in food, medicine, and agriculture.

## 5. Molecular mechanisms of TBs biological effects

TBs exert their biological activities through multiple molecular pathways, as summarized below. (i) *Signaling pathway regulation*: TBs exert metabolic effects by modulating key signaling pathways. TBs activated AMPK signaling, which promoted fatty acid oxidation through upregulation of PGC-1 $\alpha$  and CPT1 $\alpha$ , while suppressing lipid synthesis by downregulating *Srebp1c* and *Fas* expression. These effects, alongside the activation of FoxO/PPAR signaling pathway, regulate glucose and lipid metabolism and improve IR, exhibiting promising bioactivities in preventing metabolic disease.<sup>10,70,71,87,129</sup> (ii) *Apoptosis and autophagy regulation*: TBs induce apoptosis and autophagy to enhance anti-tumor effects. TBs treatment activated the mito-

chondrial apoptotic pathway by downregulating *Bcl-2* and upregulating *Bax* and *Caspase-3/9* expression, leading to mitochondrial membrane potential collapse and cytochrome C release. Simultaneously, TBs may trigger the death receptor pathway via Fas/FasL, activating Caspase-8 to further promote apoptosis. TBs also upregulated autophagy-related proteins such as LC3-II and Beclin-1, facilitating the clearance of damaged organelles.<sup>114,121,122,130</sup> (iii) *Antioxidant and anti-inflammatory mechanisms*: TBs exhibit strong antioxidant and anti-inflammatory activities through multiple mechanisms. TBs inhibited the TLR4/NF- $\kappa$ B signaling pathway, reducing the release of pro-inflammatory cytokines such as Il-6, Tnf- $\alpha$ , and Il-1 $\beta$ , and blocked the activation of the NLRP3 inflammasome. They can scavenge reactive oxygen species such as superoxide anions and hydroxyl radicals, inhibit lipid peroxidation, and enhance the activity of antioxidant enzymes including SOD, CAT, and GSH-Px, thereby maintaining cellular redox balance. In parallel, TBs modulated inflammatory responses by upregulating anti-inflammatory cytokines *Il-10* and *Tgf- $\beta$* , while suppressing pro-inflammatory factors like *Il-6* and *Tnf- $\alpha$* . They also influenced macrophage polarization by inhibiting the pro-inflammatory M1 phenotype and promoting the anti-inflammatory M2 phenotype.<sup>15,47,97,101,102,104</sup> (iv) *Intestinal microbial regulation*: TBs regulate intestinal microbial balance through both structural and metabolic mechanisms. They reshaped gut microbiota by suppressing bile salt hydrolase-associated bacteria such as *Clostridium* and *Mycobacterium* spp., while promoting beneficial butyrate-producing genera like *Akkermansia* and *Roseburia*. These shifts enhanced intestinal barrier integrity, reduced endotoxemia, and restored the F/B ratio.<sup>64,72,97,142</sup> Metabolically, TBs increased SCFAs production, which activated G protein-coupled receptors (GPR41/43) expression, thereby reducing the inflammatory response.<sup>68,122</sup> TBs also promoted the production of microbial indole pathway metabolites (such as indoleacetic acid), indirectly regulating GLP-1 secretion, and improved insulin sensitivity.<sup>91</sup> (v) *BA metabolism regulation*: TBs inhibited the ileal FXR-FGF15 signaling axis, leading to upregulation of key hepatic enzymes involved in BA synthesis, such as CYP7A1 and CYP27A1, and enhancing fecal BA excretion. This increase in hepatic BA levels exerts negative feedback on intestinal FXR signaling, forming a regulatory loop.<sup>70</sup> Additionally, TBs increased 7 $\alpha$ -dehydroxylation of BA metabolism and increased non-12OH-BAs levels including LCA and UDCA, which promoted energy metabolism and energy expenditure, illustrating the preventive roles against metabolic disease.<sup>72,134</sup> (vi) *Epigenetic regulatory mechanisms*: TBs exert epigenetic regulatory effects through both miRNA modulation and histone modification. They influenced the expression of key miRNAs such as miR-125b-5p and miR-223-3p, which in turn regulate target genes like *Sirt1* and *Ppar $\gamma$* , conferring glycolipid metabolism and inflammatory responses. TBs also modulated tumor suppressor gene expression, including p53, through miRNA-mediated DNA methylation. Additionally, TBs inhibited histone deacetylase activity, promoting chromatin remodeling and facilitating gene transcription.<sup>130,153</sup> (vii) *Other molecular mechanisms*: TBs support metabolic and



cardiovascular health through lipid regulation and vascular modulation. They reduced fat accumulation by inhibiting *Srebp1c* and *Fas* and enhanced insulin sensitivity *via* activation of the IRS-1/PI3K/Akt signaling pathway. The latest clinical studies showed that TBs promoted uric acid excretion and improved HUA by inhibiting XOD activity and upregulating uric acid transporters (OAT1/OAT3) expression.<sup>13</sup>

## 6. Conclusions

In summary, TBs are water-soluble polyphenol polymers rich in hydroxyl and carboxyl groups, with antioxidant, lipid-lowering, and intestinal microbiota regulation properties. As key quality indicators in dark tea, TBs have been demonstrated a wide range of health-promoting activities, including prevention of obesity, reduction of NAFLD and NASH, attenuation of diabetes and cancer progression, mitigation of kidney damage, alleviation of skin damage and regulation of circadian rhythms, largely through modulation of gut microbiota and BA metabolism. Despite their promising bioactivities, several challenges hinder their full development and application. First, the structural composition and biosynthetic pathways of TBs remain poorly defined. TBs are heterogeneous polymers composed of oxidized polyphenols, proteins, lipids, caffeine, and polysaccharides. This complexity limits the ability to fully characterize their structure, identify tissue-specific targets, and elucidate precise molecular mechanisms of action. The multi-functional effects observed across studies may be attributed to this structural heterogeneity. Therefore, future research should prioritize detailed structural analysis to better understand and harness TBs biological functions. The isolation methods for obtaining pure TBs from dark or black tea should be further refined to ensure a uniform composition of the extracted TBs. Illustrating the structure, even if the main composition structure will help to improve the understanding of TBs characteristics and bioactivities. Second, although TBs can be synthesized through microbial fermentation, enzymatic conversion, or mild alkali oxidation of tea polyphenols, current production methods are insufficient for large-scale industrial applications. Improvements in manufacturing processes are necessary to meet potential industrial demand. Finally, while numerous studies support the health benefits of TBs, most have been conducted using *in vitro* or rodent models. Robust clinical studies, alongside comprehensive toxicological evaluations, are warranted to confirm their safety and efficacy for long-term use. Interpreting the metabolic pathways in the body and defining the metabolites of TBs *in vitro* and *in vivo* are critically important for correlating the known bioactivities. Advancing the structural elucidation, scalable production, and clinical validation of TBs will be critical to unlocking their full potential as functional ingredients for disease prevention and human health promotion. Detailed molecular mechanism research illustrating the potent targets is recommended, accelerating food intervention of TBs supplements for weight loss or cosmetic applications. Looking ahead, future research

should prioritize the design of well-controlled clinical trials to validate the safety, efficacy, and optimal dosage of TBs in human populations, particularly for managing metabolic disorders, inflammation, and cancer risk. To support translational development, there is also strong potential for incorporating TBs into functional food products such as beverages, nutraceutical supplements, and medical foods targeting weight management, glucose control, or gut health. However, successful commercialization will require standardization of TBs isolation protocols to ensure batch-to-batch consistency in composition and bioactivity. These efforts will collectively advance TBs from a promising bioactive compound to an evidence-based ingredient in health-promoting products.

## Author contributions

Conceptualization, C. Z. and J. X.; methodology, C. Z. and Y. X.; validation, C. W., M. L., and J. X.; formal analysis, L. L.; investigation, C. Z. and Y. X.; resources, J. X.; writing – original draft preparation, C. Z., Y. X., and J. X.; writing – review & editing, M. L., H. M., and J. X.; supervision, J. X.; project administration, J. X.; funding acquisition, M. L. and J. X.

## Conflicts of interest

The authors declare no conflicts of interest.

## Abbreviations

FA	Fumaric acid
GAA	Guanidinoacetic acid
GI	Gastrointestinal
HDL-C	High-density lipoprotein cholesterol
HPLC	High-performance liquid chromatography
HUA	Hyperuricemia
IL-6	Interleukin-6
IR	Insulin resistance
LDL-C	Low-density lipoprotein cholesterol
MAPK	Mitogen-activated protein kinase
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
NF- $\kappa$ B	Nuclear factor kappa-light-chain-enhancer of activated B cells
NMR	Nuclear magnetic resonance
NO	Nitric oxide
POD	Peroxidase
PPO	Polyphenol oxidase
ROS	Reactive oxygen species
T2DM	Type 2 diabetes mellitus
TBs	Theabrownins
TC	Total cholesterol
TEM	Transmission electron microscopy
TFs	Theaflavins
TG	Triglycerides



TNF- $\alpha$	Tumor necrosis factor-alpha
TRs	Thearubigins
UV	Ultraviolet
XOD	Xanthine oxidase

## Data availability

This article is a review and does not include new experimental data. All data cited and discussed in the manuscript are derived from previously published studies, which are appropriately referenced throughout the text. No new datasets were generated or analyzed during the preparation of this work.

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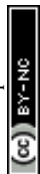
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