INTRODUCTION

Tea is popular all over the world. Green tea is a non-fermented tea containing a variety of ingredients, and the putative active principles are ascribed to polyphenols (Guo et al., 2017). Tea catechins in tea polyphenols account for more than 60% of the summation, which have been shown numerous bioactivities associated with the health-promoting actions (Zhang, Zhang, Ho, & Huang, 2019). The main phenolic compounds in green tea polyphenols (GTP) contain (-)-epicatechin (EC), (-)-epicatechin gallate (ECG), (-)-epigallocatechin (EGC), and (-)-epigallocatechin gallate (EGCG) are the most prolific active component (Zhang, Wu, Weng, & Yang, 2015). In our previous work, GTP exhibited a modulatory influence on the formation of host composition of human gut microflora, as well as the role of modulating microbial ecological imbalance and maintaining microbial ecological equilibrium (Zhang et al., 2018). Furthermore, recent researches have confirmed the function of TP in the regulation of biological clock (Xu & Lu, 2019).

The circadian rhythm means approximately 24 hr oscillated of the behavior and physiology engender via endogenous biological clock, which synchronizing numerous bioprocess with variation in environmental factors (Engin, 2017). The circadian clock system of mammalian is hierarchically organized, contains a master clock situated in the suprachiasmatic nucleus of the hypothalamus. While the peripheral clock system is mainly distributed in heart, liver, intestine, adipose, and muscle tissues (Liu et al., 2019). Circadian oscillations in physiology are driven by the circadian clock via modulating transcribe, protein abundance, and function (Noya et al., 2019). In addition, it has recently been found that the composition and function of intestinal flora also undergo...
daily oscillations. Except for the circadian clock of human, recent findings have been indicated that the gut flora also experiences daily fluctuations in formation and function (Leone et al., 2015). Disorders of circadian rhythm are considered to cause metabolic diseases including cognitive impairment (Bass & Lazar, 2016). The relation of circadian rhythm and gut microbiota has evoked widely attentions (Song, Yang, Zhang, & Wang, 2020).

The host gut microbiota is a sophisticated community, incorporating varieties of microbial flora. These microorganisms form over a long period of time symbiotic relation with human to retain metabolic equilibrium and fitness (Cheng, Zhang, Guo, Wu, & Weng, 2017). The gut has a formidable internal clock, which is critical in the function of nutrition uptake and endogenous detoxification (Plichta, Graham, Subramanian, & Xavier, 2019). Intestinal flora has been reported to participate in a variety of physiological response, containing nutrition absorption, energy modulation, dextrose metabolism, and modulation of immune system. All of these are characterized by diurnal rhythm dominate (Thaiss et al., 2014). There is evidence state clearly that the host influences the daily variations of intestinal flora via biological clock mechanism and eating habits (Kaczmarek, Musaad, & Holscher, 2017). Intestinal flora is closely related to the circadian rhythm of the host. Although there were light and dark signals, the expression of circadian clock gene was impaired in sterile mice, nevertheless the intestinal flora of normal mice showed diverse daily changes owing to dietary composition (Leone et al., 2015). Host circadian rhythm interacts with gut flora points out that new drugs have a therapeutic effect on circadian rhythm-like disorder can be prebiotics or probiotics. Consequently, the interaction between TP and intestinal flora and its effect on the circadian rhythm of host are of great significance.

As a potential functional component with circadian rhythm regulation function, natural products have drawn public attention. Plant polyphenol is a kind of highly efficient synchronizer, which has strong modulatory effects on the expression of circadian clock genes (He et al., 2016). Therefore, we attach importance to GTP as latent beneficial components, which probably conduce to the stabilization of intestinal flora and host circadian rhythms. Nevertheless, the latent mechanism of GTP mediating diurnal rhythm dysfunction is sophisticated. In this study, we used a human flora-associated mouse model to investigate the effects of GTP intervention on intestinal flora of circadian dysfunction mouse by metagenomics analysis.

2 | MATERIALS AND METHODS

2.1 | Materials

Polyamide resin was supplied by Ocean Co. Ltd. (Qingdao, China). Standards of EGC (>98%), EGCG (>98%), and ECG (>98%) were purchased from Funakoshi (Tokyo, Japan). Standard of theophylline (>98%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Standards of (-)-catechin (C) and (-)-gallocatechin-3-gallate (GCG) were intended on the basis of our reported methods (Zhang et al., 2013). Germ-free C57BL/6J mice were gained from the Experimental Animal Centre of Academy of the Military Medical Sciences (Beijing, China). All other chemicals were of analytical grade.

2.2 | Preparation of GTP

Green tea was produced in Baifeng tea garden, Beilun District, Ningbo City, Zhejiang Province. It was gathered in the crops in spring, 2019. The dried sample was grounded into powder by using a milling machine and the material that passed through a 40-mesh sieve was kept in sealed polyethylene bags at −20°C until use. In short, took 10 kg of tea powder and added 160 L of distilled water, and let them stand at 96°C for 40 min. Then, the extracting solution carried on centrifugation at 4,500 g for 15 min. According to the method we reported, the residuum has process such as dissolution, collation, and purification through polyamide column (Zhang et al., 2018). Analysis of the eluate was used high-performance liquid chromatography (HPLC) (Zhang et al., 2018). And the desired fractions were collected, concentrated, loaded onto the polyamide column, and treated as described above. As results, the fractions containing GTP were concentrated and lyophilized by a freeze-dry system.

2.3 | Animals and experimental design

All procedures involving animals were conducted at Center for Laboratory Animals, Ningbo University (Permission No. SYXX [Zhejiang] 2013-0191). We primarily colonized the young adult male mice using the microorganisms present in fresh fecal samples from six healthy volunteers who did not taken antimicrobial agents and not been admitted to a hospital within 6 months. Humanization was performed under anaerobic conditions by diluting freshly voided fecal samples (0.25 g from each donor) in 10 ml of reduced phosphate-buffered saline (PBS, 0.1 M, pH 7.2). The fecal material was then vortexed, and an aliquot of the dilution was introduced by gavage into each recipient animal. Mice were placed in darkness for sequential 7 days to adapt to the circumstances. And then, were stochastically sorted into three groups (27 mice per group): a 12 hr light-dark cycle group (control), constant darkness group (CD), and constant darkness with GTP group (GTP) fed with 0.1% (w/w) of GTP (Guo, Ho, et al., 2019). Each animal’s weight, food, and water consumption were recorded on a weekly basis. Excrementitious specimens were gathered from the GTP group after 0 (GTP-0), 2 (GTP-2), 4 (GTP-4), and 8 weeks (GTP-8). All administrative managements have been going on for 8 weeks. After the last administration, the mice were fasted for 10 hr, decapitated and killed, and blood and liver samples were taken immediately. Based on the instruction manuals, TC, TG, HDL-C, and LDL-C contents in the serum, malondialdehyde (MDA), and superoxide dismutase (SOD) in the liver were determined with the kit.
2.4 | Analysis of intestinal microbiota

DNA extraction and high-throughput sequencing were carried out according to reported methods with some modifications (Cheng et al., 2018). Stool samples were snap-frozen in liquid nitrogen before storage at −80°C. DNA from different samples was extracted using the E.Z.N.A. Stool DNA Kit (D4015, Omega, Inc., USA) according to the manufacturer’s instructions. Nuclease-free water was used as a blank. The total DNA was eluted in 50 μl of buffer and stored at −80°C until quantified by LC-Bio Technology Co., Ltd, Hangzhou, Zhejiang Province, China, and the isolation was confirmed by 1.2% of agarose gel electrophoresis.

Before sequencing, the 16S rDNA V3-V4 region of each sample was amplified with a set of primers targeting the 16S rRNA gene region. A DNA library was constructed using the TruSeq Nano DNA LT Library Preparation Kit (FC-121-4001). DNA was fragmented with dsDNA Fragmentase (NEB, M0348S) by incubating at 37°C for 30 min. Library construction began with fragmented cDNA. Blunt-end DNA fragments were generated using a combination of end-filling reactions and exonuclease activity, and size selection was performed by providing sample purification beads. Sequencing libraries were generated using the NEB Next Ultra DNA Library Prep Kit for Illumina (NEB, USA) following the manufacturer’s recommendations and index codes were added. The library quality was assessed using the Qubit@ 2.0 Fluorometer (Life Technologies, CA, USA) and Agilent Bioanalyzer 2100 system. Finally, the library was sequenced on an Illumina MiSeq platform and 300 bp paired-end reads were generated. Metagenomic sequencing was conducted using the HiSeq4000 and PE150 strategy. Pairs of reads from the original DNA fragments were merged using FLASH (V 1.2.8). RDP (V 2.11) was used to assign taxonomy to 16S rRNA gene sequencing reads. Sequences were analyzed with the Quantitative Insights Into Microbial Ecology (QIIME, V 1.8.0) software package (Caporaso et al., 2010). The high-quality reads were clustered into operational taxonomic units (OTUs) using CD-HIT software (V 4.6.1). The nucleotide similarity of OTUs is 97%, which is used for richness (Chao1) and α-diversity (Shannon and Simpson) (Schloss et al., 2009).

2.5 | Construction of intestinal metagenomic reference

The whole metagenomics analysis was based on the level of Unigene. And the metagenomics analysis was listed as below. The tool we used to predict ORFs (Open Reading Frames) was gmh-mmp (V 3.26). After removing adapters, low-quality reads and reads that belong to the host were also discarded, and then, high-quality reads were assembled to contigs using SPAdes (V 3.10.1) by employing the same parameters applied in the MetaHIT gene catalogue (Cheng et al., 2018). First, we employed the KEGG and Gene Ontology (GO) databases to perform sorting allocation and functional annotation. Apart from fitting the E value to 10^-5, employing default parameters, search all genes in the integrated microbial genome (IMG, V 3.4). The taxonomic associations of genes were decided by the minimum common ancestry of all taxonomic annotation results.

2.6 | Statistical analysis

The data collected were analyzed by SPSS software and represented by mean ± standard deviation (SD). ANOVA and Duncan’s multiple-comparison test were used in the comparison between groups. All results were considered statistically significant at p < .05.

3 | RESULTS AND DISCUSSION

3.1 | Effects of GTP on the body and organ quality of the mouse model

In our experiment, GTP was extracted from Ningbo green tea. Table 1 indicated EGCG was the dominant tea catechin in GTP.

For the variations of weight gain, compared with GTP group, the increase of CD group was higher after 2 weeks (Figure 1a), which showed the weight-reducing activity of GTP. The results of water intake demonstrated no obvious difference between the CD and GTP group (Figure 1b), and food intake showed no obvious differences in all groups, which indicated GTP did not influence the quantity of the food intake (Figure 1c). Among the peripheral clocks, the liver biological clock is one of the most crucial biological clocks. Due to the vital role in the metabolism and energy production of liver, it significantly affects the physiological state of the host (Song et al., 2020).

Research has indicated that approximately 10% of transcripts and 20% of proteins in the liver of mice were under the circadian rhythm mediation, which emphasized the potential value of the circadian clock in the liver (Reddy et al., 2006). In terms of the liver weight, CD group was evidently mitigated (p < .05) (Figure 1d). However, increased weight of liver in GTP group was evidently mitigated (p < .05) in contrast with the CD group. It indicated that CD treatment may produce an adverse effect on the circadian rhythm of the mice liver, while it can be significantly relieved after GTP intervention.

**TABLE 1** Contents of tea catechins and theophylline in the prepared GTP

<table>
<thead>
<tr>
<th>Components</th>
<th>Content (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGC</td>
<td>196.78 ± 14.38</td>
</tr>
<tr>
<td>C</td>
<td>5.66 ± 0.23</td>
</tr>
<tr>
<td>theophylline</td>
<td>0.41 ± 0.03</td>
</tr>
<tr>
<td>EGCG</td>
<td>677.14 ± 24.35</td>
</tr>
<tr>
<td>GCG</td>
<td>35.69 ± 1.64</td>
</tr>
<tr>
<td>ECG</td>
<td>161.07 ± 11.28</td>
</tr>
</tbody>
</table>
3.2 | The effect of GTP on serum and liver indicators of the mouse model

In Table S1, the elevated TG, TC, and L-DLC levels of CD group treated with GTP decreased evidently ($p < .05$). For H-DLC, comparing with CD group, GTP group had a higher level ($p < .05$). The decrease in SOD activities and increase in MDA levels in the CD group ($p < .05$) was also observed. The administration of GTP significantly enhanced SOD activity but decreased MDA level when compared with CD group ($p < .05$).

3.3 | Effects of GTP on the bacterial variety of the mouse model

The GTP treatment increased the estimated Chao1 abundance of the total diversity of the bacterial community (Table S2). Higher Shannon and lower Simpson index ($p < .05$) further supported the functional action of GTP in increasing species abundance and variety. There is a close relationship between the circadian rhythm and the gut microbiota. Study has shown that disrupted circadian clock may cause variations in the gut microbiota, containing structural composition and diurnal rhythmic oscillations (Benedict et al., 2016). As can be seen from Figure 2, Bacteroidetes, Firmicutes, and Proteobacteria were the most aplenty phyla in each group. After 8 weeks of GTP treatment, with the increase of Bacteroides, the relative abundance of Firmicutes was decreased. Relevant ratio of Firmicutes to Bacteroidetes (F/B) was declined from 0.90 (GTP-0) to 0.58 (GTP-8). The F/B ratio of the control group also declined. Nevertheless, the F/B ratio emerged the opposite trend in CD group. This suggested that the disorder of circadian rhythm probably result in the disequilibrium of gut microflora. And the results were similar to those of intestinal microecological imbalance caused by obesity. However, the occurrence of circadian rhythm disturbances involves more complex issues and cannot be simply explained by the imbalance of the F/B ratio. In order to further study the intestinal flora of mice under GTP treatment conditions, we analyzed the intestinal flora from different levels.

At the class level (Figure 3a), the richest fecal microbiota community included Bacteroidia, Clostridia, and Negativicutes. After 8 weeks of GTP intervention, the relative abundance of Bacteroidia raised from $0.50 \pm 0.02$ to $0.63 \pm 0.01$. Whereas, Clostridia and Negativicutes appertain Firmicutes appertained $F/B$ ratio revealed a contrary tendency. The main orders of GTP group were Bacteroidales, Clostridiales, and Selenomonadales. Bacteroidiales is one of the most important species, and more than 43% of the total bacteria (Figure 3b). Bacteroidales expanded (from $0.44 \pm 0.02$ to $0.59 \pm 0.03$) and an abatement in both Clostridiales (from $0.31 \pm 0.02$ to $0.24 \pm 0.02$) and Selenomonadales (from $0.12 \pm 0.02$ to $0.09 \pm 0.00$) were observed after 8 weeks GTP intervention. The change of the extensive of Bacteroidales pertaining to Bacteroidetes, Clostridiales, and
Selenomonadales pertain to Firmicutes, were in accordance with the change of phylum levels.

At the family level (Figure 3c), the abundance of Prevotellaceae and Bacteroidaceae revealed evident growth dynamic ($p < .05$) in the course of GTP treatments. In our present study, Prevotellaceae was the dominating bacterium. Its relative abundance improved from 0.34 ± 0.02 (GTP-0) to 0.43 ± 0.02 (GTP-8). The abundance of Bacteroidaceae exhibited an analogous trend. While Ruminococcaceae, Lachnospiraceae, and Veillonellaceae revealed downward trends after GTP interference for 8 weeks.

At the genus level, Prevotella and Bacteroides accounted for more than 50% of the intestinal flora (Figure 3d), whose relative abundances were raised after GTP treatment for 8 weeks. While the abundances of Mitsuokella, Faecalibacterium, and Ruminococcus were decreased.

Dyssomnia and circadian rhythm disorders are tightly associated with metabolic dysfunction. These are likely to cause obesity via disrupting the regular eating time and quantity of ingestion (Leone et al., 2015). People with continued circadian rhythm disruption have relatively high prevalence rate of hyperlipidemia, atherosclerosis, or
other diseases (Bass & Takahashi, 2010). Studies have represented that TP can regulate the comparatively shallow diurnal vibration of gene expression in the biological clock of liver, which are caused by persistent darkness (Liu et al., 2019).

The human gut is the principal site to the interactions between TP and intestinal microflora. Simultaneously, evidences have indicated an inseparable connection between intestinal flora and metabolic syndromes. Researches have emphasized the capacity of metabolites produced by microorganisms to transform diurnal rhythm and host metabolic functions (Qi et al., 2017). Our previous investigation demonstrated the change of intestinal microbiota composition is associated with the metabolic disorders (Cheng et al., 2017). TP and its aromatic metabolites have antibacterial or bactericidal effects, and can restrain bacterial adhesion. They may have a positive influence on the intestinal microenvironment by regulating the number and structure of bacteria (Lee, Jenner, Low, & Lee, 2006).

In our previous studies, tea catechins dramatically accelerated the proliferation of *Bifidobacterium* spp., which is conventionally considered as a probiotic (Zhang et al., 2013). Furthermore, the content of short-chain fatty acid (SCFAs) in tea catechin culture was higher than that of other substances. It is indicated that TP may be appropriate substrates for certain gut flora. These results are consistent with the regulatory effect of GTP on gut flora in diet-induced obese mouse model (Guo et al., 2017; Zhang et al., 2018). The microbiome has been indicated to modulate gut absorption and fatty acid metabolism. For SCFAs, especially acetate, only a small part is metabolized in the intestine, and the rest reaches the liver through the portal vein to participate in the metabolism (Velumurugan et al., 2017). In addition, our previous studies indicated TP may improve the intestinal flora imbalance caused by circadian rhythm disorder and influencing metabolic pathways, ameliorating the improvement of host microecology (Guo et al., 2017). TP can also alleviate the circadian oscillations and phase shifts of clock genes in the intestine and liver of mice induced by CD treatment (Guo et al., 2019).

### 3.4 | Effects of GTP on the intestinal microbiome of the mouse model

To describe the functional gene products, GO database supplies three forms of system definitions. We chose 10 of the maximum GO items in each function. GO analysis of differentially expressed genes (DEGs) between GTP-0 and GTP-8 showed that the DEGs in the field of the bioprocess were primarily “biological process,” “metabolic process,” and “proteolysis.” In the aspect of cellular constituent, the principal concentrated GO terms contained “cytosol,” “cellular component,” and “cytoplasm.” In the territory of molecular function, “molecular function,” “metal ion binding,” and “hydrolase activity” were the principal groups (Figure 4).

Now in this research, KEGG analysis of DEGs exhibited the most abundant metabolic pathway between GTP-0 and GTP-8. Biosynthesis of amino acids, two-component system, and ATP-binding cassette (ABC) transporters took up the top categories (Table S3), showing that GTP treatment has a striking effect on these pathways. We also used KEGG analysis to classify these genes into different categories (Figure 5), and the DEGs were participated in pathways for instance ubiquinone and other terpenoid-quinone biosynthesis, pentose and...
glucoronate interconversions, and lipopolysaccharide biosynthesis and terpenoid backbone biosynthesis.

Microorganisms can upregulate proteins associated with defensive mechanism, which can defend cells and concurrently downregulating various metabolism and biosynthesis proteins related to them (Cheng et al., 2018). Trillions of microorganisms make up the intestinal flora, they contained huge and rare metabolic mechanisms of xenobiotics. Physiological changes in the gut microbiota affected the metabolism of the host, thus, determined the transition between health and disease (Velmurugan et al., 2017). At present, the genes of intestinal bacteria have been determined by direct metagenomics sequencing, and the metabolic potential of the selected gene pool in intestinal bacteria has been understood (Turnbaugh et al., 2009). The formation and function of intestinal microbiota change with the diurnal variation of diet through the regulation of host circadian rhythm. Moreover, microbial communities play a key part in preserving host circadian rhythm and metabolic equilibrium. Through the correlations between host circadian rhythm and intestinal flora reveal that prebiotic or probiotic intervention is a feasible method to relieve circadian rhythm misalignment and associated metabolic diseases (Song et al., 2020).

Recent studies have also confirmed that microbial metabolic derivatives can regulate the circadian rhythms of the central and liver as well as metabolic functions of the host, which means prebiotics, can alleviate circadian rhythm disorders (Tahara et al., 2018). In this study, through the GO analysis of DEGs, we found that most of the genes in cell constituents contain cytosol, cytoplasm, and cell membrane. These indicating that GTP intervention probably influence these cell constituents, so as to reduce the adverse consequences of CD induction. In the analysis of KEGG pathway of DEGs between GTP-0 and GTP-8, amino acid biosynthesis, two-component system, and ABC transporter occupy the first several categories, which illustrating that GTP treatment has an evident impact on these pathways.

According to the report, EGCG can alleviate the metabolic syndrome related to biological clock caused by diet (Mi et al., 2017). Dietary TP can improve memory impairment through biological clock relevant mechanism (Qi et al., 2017). Nevertheless, due to the limited bioavailability in vivo, most of the TP ingested is metabolized into multifarious ramifications by gut flora. The activity of TP depends to a considerable degree on their transformation in the gut. Studies have shown that disorders in circadian cycle of light and dark induced change in gut microbiome, which involved composition and circadian oscillations (Deaver, Eum, & Toborek, 2018). The effect of gut microflora in regulating circadian rhythm by TP is sophisticated. The mechanism of the interaction...
between TP and intestinal microbiota and its implication for the prevention of circadian rhythm-related metabolic diseases need further investigation in the future.

4 | CONCLUSIONS

In this study, GTP effectively ameliorated circadian rhythm disorder-induced gut microecology imbalance. The results demonstrated that the GTP intervention may be beneficial to the stabilization of some intestinal flora. KEGG analysis showed that in order to cope with the pressure of circadian rhythm dysfunction, genes relevant to ubiquinone, other terpenoid-quinone biosynthesis, pentose and tautomeric glucurionate, and lipopolysaccharide biosynthesis were dramatically differentially expressed. Eventually, our results stated clearly that GTP can be developed to a satisfied source of resistance to metabolic syndrome caused by circadian rhythm disorder.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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