Systematic Review

Effects of Polyphenols in Tea (Camellia Sinensis sp.) on Modulation of Gut Microbiota in Human Trials and Animal Studies

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Abstract: A diet high in polyphenols is associated with a diversified gut microbiome. Tea is the second most consumed beverage in the world, after water. The health benefits of tea might be attributed to the presence of polyphenol compounds such as catechins, theaflavins, tannins, and flavonoids. Although many studies are on tea, little is known of its effects on trillions of gut microbiota. Hence, this review is aimed at systematically studying the effect of tea polyphenols on the stimulation or suppression of gut microbiota in humans and animals. It was conducted according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) protocol. Articles were retrieved from PubMed and Scopus databases, and data were extracted from 6 human trials and 15 animal studies. Overall, huge variations were observed in terms of microbiota composition between humans and animals. A more consistent pattern of diversified microbiota was observed in animal studies. Tea alleviated the gut microbiota imbalance caused by high-fat diet-induced obesity, diabetes, and ultraviolet-induced damage. Overall changes in microbiota composition measured by beta diversity analysis showed that tea had shifted the microbiota from the pattern seen in animals that received tea-free intervention. In humans, the prebiotic-like effect was observed towards gut microbiota, but these results appear in lower-quality studies. Beta diversity in human microbiota remains intact despite tea intervention; supplementation with different teas affected different types of bacterial taxa in the gut. These studies suggest that tea polyphenols may have a prebiotic effect in disease-induced animals and in a limited number of human interventions. Further intervention is needed to identify the mechanisms of action underlying the effects of tea on gut microbiota.

Keywords: Camellia sinensis; tea polyphenols; gut microbiota; gastrointestinal bacteria; systematic review

1. Introduction

Studies on the relationship between gut microbiota and health have garnered much interest in recent years. The term “gut microbiota” is defined as the microbial ecosystem or community that resides within the human intestinal tract [1]. The gut ecosystem comprises microorganisms, mainly bacteria, and a small number of viruses, protozoa, and eukaryotic organisms such as fungi that are distributed throughout the entire gastrointestinal tract [2]. As stated by Nahoum et al. (2016), diversified microbiota are a crucial indicator of good health and well-being [3].

Gut microbiota play an important role in human health, and are considered a “forgotten organ” and “super-organism” that maintains intestinal epithelium integrity [4–6]. The human gut contains an estimated 100 trillion microorganisms [7]; in addition, over 1000 different species of microbes colonize the human gut [8]. The dominant groups of bacteria phyla in the gut are Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria [5, 9]. Fusobacteria, Cyanobacteria, and Verrucomicrobia phyla are usually less well-represented [10].

Gut microbiota may also play a role in how drugs are metabolized in our body [11]. These commensal bacteria play an important role as key regulators of digestion involving the extraction, synthesis, and absorption of many nutrients and metabolites, including bile
acids, lipids, amino acids, vitamins, and short-chain fatty acids (SCFAs) [11]. Gut microbiota also have a crucial immune function against pathogenic bacteria colonization through many competition processes [12]. They inhibit pathogenic bacteria growth by consuming available nutrients, pH modification, and producing bacteriocins, which are a type of antimicrobial peptide secretion, and affect cell signaling pathways [13].

Gut microbiota imbalance (dysbiosis) is associated with the development of a series of diseases [15–20]. These diseases include diabetes, obesity, cardiovascular and liver diseases, cancers, multiple sclerosis, and neurodegenerative diseases [14–19]. Hence, gut microbiota have been proposed as a promising therapeutic target for these diseases [20].

Research is still ongoing on the factors that modulate gut microbiota profiles, and diet could play a role [21–23]. However, studies suggest that diet only accounts for a low percentage of microbiome variation after adjusting for other contributing co-variates, such as genetics, age, ethnicity, geographic origins, body mass index (BMI), lifestyle, medication, and environmental factors [12, 24].

Dietary intake varies from one individual to another, and there is a complex interaction between dietary intake and the gut microbiome [25]. The so-called “Western diet” is characterized as high-calorie, and is associated with obesity, coronary vascular disease, and metabolic syndrome [2]. Evidence suggests that the so-called “Mediterranean diet”, which is high in the polyunsaturated fatty acids and polyphenols (from coffee, tea, or grapes) associated with increased gut microbiota diversity [26].

Tea (Camellia sinensis sp.) is one of the most widely consumed non-alcoholic beverages in the world [27]. There are different types of tea: namely green tea, oolong tea, black tea and dark tea. Each tea is produced differently using different fermentation processes. Tea is rich in polyphenols, possesses antioxidant properties, and offers multiple health benefits [28]. Flavonoids such as flavanols (catechins, galloatechin, epicatechins), flavonols (kaempferol and quercetin), and phenolic acids are the three major classes of polyphenols in tea [29].

Polyphenols are absorbed in the small intestine and may reach the colon [30]; these polyphenols are metabolized by gut microbiota into metabolites such as phenolic acids [31]. In vitro studies showed that polyphenols in tea are biotransformed to active metabolites by gut microbiota through enzymatic activities and the increased bioavailability of polyphenols [32, 33]. Polyphenols such as flavanols including catechins modulate the composition of the gut microbial community, mostly through the inhibition of pathogenic bacteria and the stimulation of beneficial bacteria species such as Bacteroides galacturonicus, Lactobacillus sp., Enterococcus caccae, Bifidobacterium catenulatum, Ruminococcus gauvreauii, and Escherichia coli [33, 34]. Previous studies showed that the synergistic effect of tea polyphenols and gut microbiota has subsequently influenced the host biochemical processes, establishing a system of mutual interaction and interdependency [35]. The presence of polyphenols could increase the host immune system and metabolic responses through the modulation of gut microbiota [35].

The evidence suggests that polyphenols may also modulate gut microbiota in what is known as a prebiotic-like effect [20]. However, Ivey et al. (2019) reported that dietary flavanols produced a vast potential complexity of interactions when combined with the phylogenetic and functional diversity of the human gut microbiota [36]. This complexity is linked to the capacity of flavanols to promote beneficial bacteria or suppress pathogenic bacteria [37–39].

To the best of our knowledge, the effects of tea on gut microbiota were studied in cells (in vitro) and in mechanistic studies on animal models (in vivo) [40–43]. However, studies using cell lines or animal models to study gut microbiota have their own limitations. Casotta et al. (2020) showed that findings from animal models and cell culture do not represent and are not translatable to humans [44]. The main limitation of in vitro studies is due to the host’s tolerance of microbial infections, which varies greatly across different species [45]. In vitro colonic fermentation models are cheaper, more reproducible, and can be conducted in a shorter time compared with in vivo studies [46]. Pham et al.
(2018) showed several limitations of cell studies, including the absence of human or animal cells and low pH which reduces microbial activity [46].

Furthermore, it remains a challenge to translate findings obtained from cells and animal models to humans [47]. The role of polyphenols in tea in modulating human gut microbiota is not well understood. This highlights the need and importance of standardizing human studies, and better outcomes could be predicted. It is still too unclear to suggest an effective dose, choice of types (green, oolong, black or dark tea) or forms (liquid, powder, extract), and the duration of tea intake needed to increase the diversity of gut microbiota in humans. Therefore, this systematic review was aimed at contributing to current updated evidence and knowledge on tea polyphenol stimulation or suppression of the diversity in gut bacteria population in humans and animals. The next aim was to determine the effective types of tea (green, black, oolong, or dark tea), dosage, tea forms (liquid, powder, pure extract) and duration of intake to modulate gut microbiota.

2. Methods

Search strategy

Studies were selected using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Two search databases, namely PubMed and Scopus, were used to search articles published between the years 2000 and 2020. The Boolean operator term AND was used to focus and narrow the search, while OR was used to expand the search by linking synonyms. The following key terms were applied during the search:

1. (Tea) AND (Gut Microbiota OR Gut Microbiome OR Gut Microflora)
2. (Tea Polyphenol OR Tea Catechin) AND (Intestinal Microbiota OR Intestinal Flora)
3. (Caffeinated Tea OR Decaffeinated Tea) AND (Colon Microbiota OR Colon Microbial)
4. (Green Tea OR Black Tea OR Oolong Tea) AND (Gut Bacteria OR Enteric Bacteria)
5. (Tea OR *Camellia sinensis* sp.) AND (Gastrointestinal Microbiota OR Gastrointestinal Bacteria)
6. (Tea) AND (Firmicutes OR Bacteroidetes OR Actinobacteria OR Proteobacteria)
7. (Flavonoids OR Flavanols OR Flavonols OR Phenolic acids) AND (Gut Microbiota OR Gut Microbiome OR Gut Microflora)
8. (Catechin OR Galallocatechin OR Galallocatechin gallate OR Epicatechin OR Epicatechin gallate OR Epigallocatechin OR Epigallocatechin gallate) AND (Gut Microbiota OR Gut Microbiome OR Gut Microflora)
9. (Catechin OR Galallocatechin OR Galallocatechin gallate OR Epicatechin OR Epicatechin gallate OR Epigallocatechin OR Epigallocatechin gallate) AND (Gut Microbiota OR Gut Microbiome OR Gut Microflora)

Study selection

Two authors independently screened the articles and extracted the data. Jadad scoring was used to assess the risk of bias in human trials. The lowest possible score is 1, while the highest possible score is 5 (indicating the highest quality human trials) [49]. Studies were qualified for eligibility according to pre-specified inclusion criteria. The inclusion criteria were: 1) English primary research paper published between 2000 to 2020; 2) Papers on randomized control trials and *in vivo* studies; 3) Studies with normal or overweight (BMI of 18.5 – 29.9) subjects, non-smokers and non-drinkers, free from medications or supplements; 4) Subjects who have had a low-polyphenol diet before enrolling into intervention; 5) All subjects given *Camellia sinensis* tea and compared with
placebo and/or no treatment; and 6) Study outcomes measuring gut microbiota diversity, including alpha diversity (richness, evenness, relative abundance) and beta diversity (overall bacteria composition).

3. Results

A total of 5,671 articles on human trials and in vivo animal studies were retrieved from the preliminary search using Scopus and PubMed. Duplicate articles (n = 2,663) were removed and the remaining 3,008 articles were screened for the relevant title and abstract. A total of 2,963 non-relevant articles were further excluded, and the remaining 45 articles were screened for full content. Twenty-four articles did not meet the inclusion criteria and were excluded. Among those, fifteen studies were excluded because they used multi-component tea supplements in the intervention. Eight studies focused on urinary metabolites of gut microbiota rather than the composition of the commensal microorganism and were thus excluded. One randomized control trial described only intervention protocols and hence was excluded. A total of 6 human trials and 15 animal studies were included in the final qualitative review (n = 21); Figure 1 shows the PRISMA flow diagram. Human studies showed a “high” risk of bias as assessed by Jadad score (Table 1).

![Figure 1. PRISMA flow diagram used to identify human trials and animal studies.](image)

![Table 1. Risk of bias assessment based on Jadad score.](image)
<table>
<thead>
<tr>
<th>Reference</th>
<th>Jaded scores</th>
<th>Study quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zhou et al. (2019) [49]</td>
<td>5</td>
<td>High</td>
</tr>
<tr>
<td>Mai et al. (2004) [50]</td>
<td>5</td>
<td>High</td>
</tr>
<tr>
<td>Janssens et al. (2016) [51]</td>
<td>4</td>
<td>High</td>
</tr>
<tr>
<td>Yuan et al. (2018) [52]</td>
<td>0</td>
<td>Low</td>
</tr>
<tr>
<td>Huang et al. (2019) [53]</td>
<td>0</td>
<td>Low</td>
</tr>
<tr>
<td>Jin et al. (2012) [54]</td>
<td>0</td>
<td>Low</td>
</tr>
</tbody>
</table>

3.1. Green tea and gut microbiota

Green tea is processed swiftly using fresh leaves to prevent fermentation [28]. Thus, the polyphenol content is higher in green tea compared with other types of tea [28]. Tables 2 and 3 summarize the findings on four human trials and five animal studies on green tea modulation effects and gut microbiota.

No changes were observed in gut microbiota from a high-quality clinical trial administering four decaffeinated green tea capsules daily containing 1315 ± 115.0 mg of catechins in post-menopausal women for one year [49]. Another trial observed the same results, except for the fact that overweight subjects showed a lower microbiota diversity compared with normal-body weight subjects before the intervention [51]. Yuan et al. (2018) found that tea reversed the gut microbiota patterns seen in patients with colorectal cancer [55-58]. However, it must be noted that Yuan et al. (2018) showed a low Jadad score for study quality. Another study found increased levels of bacteria responsible for producing short-chain fatty acids (the main energy source of cells in gut lumen) after receiving 400 mL of green tea beverage per day (approximately two cups daily) for two weeks [52]. Jin et al. (2012) found an increase in probiotic *Bifidobacteria* when the subjects replaced their water with green tea liquids for ten days [54].

The effects of green tea in animal models were consistent. Mice were given different stressors to cause dysbiosis (imbalance) in their gut microbiota. Zhang et al. (2020) induced diabetes alloxan-injection into the mice and supplemented their diet with tea for one month [59]. Diabetes had shifted all diversity measures of the microbiota, and incorporating tea in the diet lowered the indexes to levels almost similar to those in normal mice [59]. Wang et al. (2018) administered tea as drinking water along with a high-fat diet in human flora-associated mice for eight weeks [60]. Tea reversed all changes induced by obesity, hence increasing the overall microbial diversity [60]. Wang et al. (2016) supplemented green tea in a high-fat diet and showed an increased abundance of beneficial lactic acid bacteria (*Lactobacillus* sp.) [61]. Jung et al. (2017) exposed the mice to chronic ultraviolet rays which subsequently changed the dominant phylum of microbiota [62]. Receiving tea extract for 10 weeks completely reversed the changes induced in the mice by the ultraviolet rays [62]. Seo et al. (2015) found a significant reduction in biomarkers of obesity and insulin resistance (ratio of *Firmicutes* to *Bacteroidetes* phyla and ratio of *Bacteroidetes* to *Prevotella* phyla) in the high-fat diet group after intubating tea extracts orally for eight weeks [63].

3.2 Oolong tea and gut microbiota

Oolong tea is also known as “semi-fermented” or “partially oxidized” tea. Catechins in oolong tea are oxidised into theaflavins, thearubigins, and theabrownins during partial fermentation, hence producing a slightly darker color than green tea [64]. Oolong tea was supplemented in two murine studies (Table 3). Studies by Cheng et al. (2018) and Cheng et al. (2017) investigated the effects of oolong tea extracts in mice induced with human
flora and given a high-fat diet [40, 42]. Tea increased gut microbiota diversity after four to eight weeks of tea supplementation [40, 42].

3.3 Black tea and gut microbiota

Black tea is a “fully fermented” tea and is characterized by a darker color and astringent taste due to a higher concentration of theaflavins, thearubigins, and theabrownins compared to other types of tea [64, 65]. Polyphenol oxidase is a heat-labile enzyme present in black tea [65]. The activity of this enzyme is reduced by steam-heating during the fermentation of black tea, and consequently reduced their antioxidant properties compared to green tea [65, 66]. In this review, one human study demonstrated the effect of black tea on the gut microbiota (Table 3). Black tea infusion was given to hypcholesterolemic volunteers in a double-blind, randomized crossover feeding trial for six weeks [50]. However, no significant changes were observed in the gut microbiota [50].

3.4 Pu-erh tea and gut microbiota

Pu-erh tea is a traditional Chinese tea. There are two types of Pu-erh tea, namely raw (unfermented) and ripe (after microbial fermentation) [67]. In this review, one human trial and four murine studies were done on Pu-erh tea (Table 2 and Table 3). Huang et al. (2019) investigated the cholesterol-lowering activity of ripe Pu-erh tea in humans and animals [53]. In this study, male human subjects received 600 mL of tea infusion (approximately three cups) daily for four weeks, while the mice were provided with a daily dose of 450 mg of tea extracts per kg body weight in a high-fat diet for 26 weeks [53]. Hyper-cholesterol enriching bacterial genera were significantly reduced compared to high-fat diet numbers in human and animal studies [53]. Three murine studies demonstrated the effects of raw and ripe Pu-erh tea in restoring the altered gut microbiota caused by a high-fat diet. Lu et al. (2019) and Xia et al. (2019) showed that Pu-erh tea at a dose between 0.1 g to 0.4 g of tea extracts for five to eight weeks effectively increased gut microbiota diversity [68, 69]. Gao et al. (2017) found that ripe Pu-erh tea extract and Pu-erh tea polyphenol components increased gut microbiota diversity in the high-fat diet group [70].

3.5 Fuzhuan tea and gut microbiota

Fuzhuan brick tea is a type of dark tea known as fungal fermented tea [71]. The polyphenol content in Fuzhuan tea is lower compared to green tea, due to the process of microbial fermentation occurring in dark tea production [72, 73]. A series of reactions, including degradation, oxidation, condensation, structural modification, methylation, and glycosylation, are catalyzed by microbial exo-enzymes or occur as a result of microbial metabolism, leading to the development of dark tea quality [74–76]. Studies by Chen et al. and Foster et al. incorporated two different dosages of Fuzhuan tea extracts in mice receiving a high-fat diet (Table 3) [41, 77]. Daily supplementation of Fuzhuan tea extracts at the dose of between 200 to 400 mg for eight weeks was able to reverse the altered dominant phyla bacteria in the gut and also increased the levels of Lactobacillus and Bifidobacteriaceae [41, 77].

3.6 Multiple types of tea and gut microbiota

Two murine studies compared the modulating effects of multiple teas on gut microbiota that were exposed to a high-fat diet (Table 3) [78, 79]. Henning et al. (2017) showed that supplementation of 0.5 g decaffeinated green and black tea extract daily for four weeks increased the level of phylum Bacteroidetes while suppressing phyla Firmicutes and Actinobacteria. The ratio of Firmicutes to Bacteroidetes was also reduced [79]. Liu et al. (2016) monitored the effects after feeding 100 mL of either green, oolong, or black tea liquid daily for 13 weeks, and noted a reversed trend in the growth of bacteria, compared to those with only a high-fat diet [79].
<table>
<thead>
<tr>
<th>Reference</th>
<th>Subjects</th>
<th>Dose, duration</th>
<th>Alpha diversity</th>
<th>Beta diversity</th>
<th>Key findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zhou et al. (2019)</td>
<td>124 post-menopausal females</td>
<td>Four green tea pills/day (1315 ± 115.0 mg catechins) for 12 months</td>
<td>No change</td>
<td>No change</td>
<td>No change in Firmicutes, Bacteroidetes, Actinobacteria</td>
</tr>
<tr>
<td>Country: United States</td>
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<tr>
<td>Yuan et al. (2018)</td>
<td>12 healthy normal and overweight males and</td>
<td>400 mL/day (100.2 μg GAE/mL of total polyphenols) for 2 weeks</td>
<td>Increased</td>
<td>Increased</td>
<td>↓Bacteroidetes, ↑Firmicutes, ↑Actinobacteria</td>
</tr>
<tr>
<td>Country: China</td>
<td>females</td>
<td></td>
<td></td>
<td></td>
<td>↑FIR:BAC</td>
</tr>
<tr>
<td>Janssens et al. (2016)</td>
<td>58 Caucasian normal to overweight males and females</td>
<td>Nine green tea pills/day (0.56 g of EGCG) for 12 weeks</td>
<td>No change</td>
<td>No change</td>
<td>No change in Firmicutes, Bacteroidetes, Actinobacteria, Fusobacteria</td>
</tr>
<tr>
<td>Country: United States</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Verrucomicrobia</td>
</tr>
<tr>
<td>Jin et al. (2012)</td>
<td>10 non-habitual male and female tea drinkers</td>
<td>1000 mL green tea/day (unknown amount of polyphenols) for 10 days</td>
<td>Not measured</td>
<td>Not measured</td>
<td>↑Bifidobacteria</td>
</tr>
<tr>
<td>Country: Japan</td>
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<tr>
<td>Mai et al. (2004)</td>
<td>8 hypercholesterol subjects</td>
<td>Black tea infusion (unknown dose and polyphenol)</td>
<td>Not measured</td>
<td>Not measured</td>
<td>No changes in Bacteroides, Prevotella Faecalibacterium</td>
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<tr>
<td>Reference</td>
<td>Dose, duration</td>
<td>Alpha diversity</td>
<td>Beta diversity</td>
<td>Key findings</td>
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<tr>
<td><strong>Zhang et al. (2019) [59]</strong></td>
<td>0.1 g of matcha powder (14% tea polyphenols, 4.5% EGCG) or instant green tea (22.7% tea polyphenols, 8.4% EGCG) per 100g of diet for 30 days</td>
<td>Increased</td>
<td></td>
<td></td>
<td>Tea increased diversity of microbiota, reversing the changes caused by diabetes.</td>
</tr>
<tr>
<td></td>
<td><strong>Wang et al. (2018) [60]</strong></td>
<td>0.05, 0.2, 0.8 g green tea extract per 100 mL of water (contains 804 mg/g of total catechins, 455</td>
<td>Not measured</td>
<td></td>
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</table>

FIR:BAC = ratio of Firmicutes to Bacteroidetes phyla; EGCG = Epigallocatechin-3-gallate.
<table>
<thead>
<tr>
<th>Study</th>
<th>Intervention</th>
<th>Microbial Changes</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jung et al. (2017) [62]</td>
<td>1 g extract/100 g diet (contains 50% of total catechins) for 10 weeks</td>
<td>↓ Clostridium</td>
<td>Tea increase diversity of microbiota, reversing the changes caused by chronic ultraviolet exposure</td>
</tr>
<tr>
<td>Wang et al. (2016) [61]</td>
<td>0.05, 0.2, 0.8 g of green tea polyphenol compound per 100 g of HFD for 8 weeks</td>
<td>Increased, ↑ Firmicutes, ↑ Bacteroidetes, ↑ Proteobacteria</td>
<td>Tea increased beneficial Lactobacillus</td>
</tr>
<tr>
<td>Seo et al. (2015) [63]</td>
<td>500 mg of fermented green tea extract/kg in HFSD (contains 7.85% catechins) for 8 weeks</td>
<td>Not measured, Not measured, ↓ FIR:BAC, ↓ BAC:PREV</td>
<td>Tea reduced biomarkers of obesity and insulin resistance</td>
</tr>
<tr>
<td>Cheng et al. (2018) [42]</td>
<td>0.1 g of oolong tea polyphenols per 100 g of HFD (contains 43.55 ± 3.77 µg/g of EGCG) for 4 weeks</td>
<td>Increased, ↓ Firmicutes, ↑ Bacteroidetes, ↑ FIR:BAC, ↑ Proteobacteria</td>
<td>Tea increased diversity of microbiota, reversing the changes caused by obesity</td>
</tr>
<tr>
<td>Cheng et al. (2017) [40]</td>
<td>0.1 g oolong tea polyphenols (EGCG) per 100 g of HFD for 8 weeks</td>
<td>Increased, ↓ Firmicutes, ↑ Bacteroidetes, ↑ FIR:BAC, ↑ Proteobacteria</td>
<td>Tea increased diversity of microbiota, reversing the changes caused by obesity</td>
</tr>
<tr>
<td>Huang et al. (2019) [53]</td>
<td>450 mg/kg/day of ripe Pu-erh tea extracts in HFD (containing 52.75% theabrownin) for 26 weeks</td>
<td>Not measured, Not measured, ↓ Bacilli, ↓ Lactobacillus, ↓ Enterococcus, ↓ Lactococcus, ↓ Streptococcus</td>
<td>Pu-erh tea reduced diversity of hyper-cholesterol enriching bacteria</td>
</tr>
<tr>
<td>Lu et al. (2019) [68]</td>
<td>0.1 g, 0.2 g and 0.4 g of ripe Pu-erh tea extract per 100 mL water in HFD</td>
<td>Increased, Increased, ↓ FIR:BAC, ↑ Anaerotruncus, ↑ Alistipes, ↑ Odoribacter</td>
<td>Tea increased diversity of microbiota, reversing the changes caused by obesity</td>
</tr>
<tr>
<td>Study</td>
<td>Treatment and Duration</td>
<td>Microbiota Changes</td>
<td>NotMeasured</td>
</tr>
<tr>
<td>---------------------------</td>
<td>----------------------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------------</td>
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</tr>
<tr>
<td>Xia et al. (2019) [69]</td>
<td>0.15-g and 0.4-g extracts/kg body-weight of raw Pu-erh tea (2.73± 0.28 % of catechin) and ripe Pu-erh tea (contains 0.56 ± 0.07 % of catechin) in HFD for 5 weeks</td>
<td>Increased Firmicutes, Increased Blautia, Increased Roseburia, Increased Bacteroides, Increased Parabacteroides, Increased Akkermansia, Increased Bilophila, Decreased Leuconostoc, Decreased Allobaculum</td>
<td>Not measured</td>
</tr>
<tr>
<td>Gao et al. (2017) [70]</td>
<td>750 mg/kg of ripe Pu-erh tea extract and 250 mg/kg of Pu-erh tea polyphenol and oxidized tea polyphenol in HFD for 12 weeks</td>
<td>Not measured Firmicutes, Not measured Bacteroidetes, Not measured Eubacterium rectale, Not measured Clostridium cocoides, Not measured Faecalibacterium prausnitzii, Not measured Akkermansia muciniphila, Not measured Bifidobacterium, Not measured Lactobacillus, Not measured Roseburia</td>
<td>Not measured</td>
</tr>
<tr>
<td>Chen et al. (2018) [41]</td>
<td>400 mg/kg/day of Fuzhuan tea extract (contains 26.05 ± 1.15% polyphenols) in HFD for 8 weeks</td>
<td>Not measured Firmicutes, Increased Bacteroidetes, Increased FIR:BAC, Increased Proteobacteria, Increased Bifidobacteriaceae</td>
<td>Changed</td>
</tr>
<tr>
<td>Study</td>
<td>Amount of Tea</td>
<td>Phyla Changes</td>
<td>Duration</td>
</tr>
<tr>
<td>----------------------</td>
<td>------------------------------------------</td>
<td>------------------------------------------------------------------------------</td>
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</tr>
<tr>
<td>Foster et al. (2016)</td>
<td>1400 mg/kg/week of Fuzhuan tea extract</td>
<td>↓Firmicutes, ↑Bacteroidetes, ↑Lactobacillus</td>
<td>8 weeks</td>
</tr>
<tr>
<td></td>
<td>(unknown polyphenol amount) in HFD for 8 weeks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Henning et al. (2017)</td>
<td>0.5 g of decaffeinated green tea extract (contains 565 ± 24 GAE mg/g) or black tea extract (contains 532 ± 25 GAE mg/g) per 100 g of HFD for 4 weeks</td>
<td>↑Bacteroidetes, ↓Firmicutes, ↓Actinobacteria, ↑FIR:BAC, ↑Parabacteroides, ↑Clostridium, ↑Coprococcus, ↑Pseudobutyribrio</td>
<td>Changed</td>
</tr>
<tr>
<td>Liu et al. (2016)</td>
<td>100 mL tea infusion of green tea (contains 3332.35 ± 70.91 mg/L of total polyphenols), oolong tea (contains 2911.52 ± 51.51 mg/L of total polyphenols) and black tea (contains 2732.11 ± 23.64 mg/L total polyphenols) in HFD for 13 weeks</td>
<td>↑Alistipes, ↑Rikenella, ↑Lachnospiraceae, ↑Akkermansia, ↑Bacteroides, ↓Parabacteroides</td>
<td>Increased</td>
</tr>
</tbody>
</table>

Total phenolic content was expressed as GAE
GAE = Gallic acid equivalent; HFD = High fat diet; EGCG = Epigallocatechin-3-gallate; HFSD = High fat sugar diet; FIR: BAC = ratio of Firmicutes to Bacteroidetes phyla; BAC: PREV = ratio of Bacteroidetes to Prevotella
4. Discussion

Gut microbiota are known for their large variations in terms of taxonomy and functionality [12]. Each individual has a unique gut microbiota profile that differs from another’s [12]. Genetic and environmental factors have directly influenced gut microbiota composition [80]. In terms of genetics, gut microbiota can be shaped according to birth gestational age, type of birth delivery, methods of milk-feeding, and weaning period [12]. The composition of gut microbiota also differs greatly due to many lifestyle-associated factors, including dietary choices, physical activity, body mass index (BMI), age, food additives and contaminants, and antibiotic consumption, that indirectly shape the gut microbiota composition [24, 81].

This review showed that *Camellia sinensis* could modulate the gut microbiota. Overall, 3 human studies and 15 animal studies from a total of 21 included in the review showed a significant increase in diversity of gut microbiota. Most animal studies were able to reverse the disrupted microbiota changes due to stressors such as diabetes, obesity and ultraviolet ray damage. Beta diversity measured in murine studies shows an overall shift in the mice gut microbiota profile after tea supplementation. This indicates that the modulatory effects of tea were attributable to its ability in mediating specific imbalances in the gut. Three out of six human trials showed diversified microbiota as a result of incorporating tea [52–54]. An increase in the richness, evenness and relative abundance of beneficial bacteria and a reduction of non-beneficial bacteria were observed in the studies [52–54].

Green tea is the main type of tea used in this review. An average of two to five cups of green tea per day for 10 days and up to two weeks was associated with increased beneficial probiotic *Bifidobacteria* and their colon cancer-preventative properties in humans [52, 54]. Colon microbiota have the ability to metabolize tea polyphenols into short-chain fatty acids (SCFA) and phenolic acids, before being metabolized in the liver or being excreted [82]. A previous *in vitro* study showed that black tea prepared in bread had no impact on short-chain fatty acid (SCFA) production [83].

In a low-quality human trial, green tea increased clusters of bacteria specializing in producing short-chain fatty acids (SCFA), namely *Lachnospiraceae, Ruminococcaceae, Dorea, Roseburia, Faecalibacterium, Eubacterium, Blautia*, and *Coprococcus* [52]. Short-chain fatty acids are a primary energy source for colonic epithelium cells, as they maintain intestinal homeostasis through anti-inflammatory actions [84, 85]. With elevated fecal SCFA concentrations, SCFA-producing bacteria may promote reduced inflammation in the gut [84, 85]. This might be important in the preventative steps against colorectal cancer, since inflammatory bowel disease patients showed reduced levels of dominant SCFA-producing bacteria in several studies [86–90]. However, further study is needed to determine whether green tea could possibly modulate gut microbiota in cancer patients.

Daily Pu-erh tea intakes of 600 mL (around three cups) for four weeks reduced the proliferation of hypercholesterol-enriching bacteria (*Bacilli, Clostridia, Lactobacillus, Bacillus, Streptococcus, and Lactococcus*) [53]. These bacteria are involved in bile acid metabolism, i.e. to generate bile salt hydrolase (BSH) enzymes that reduce cholesterol level [53]. Obese human subjects showed a higher *Firmicutes/Bacteroidetes* ratio after supplementation with polyphenols, and this has been proposed as a reason for weight loss [91, 92]. A previous study showed that body weight and dramatic dietary patterns might affect gut microbiota composition [93, 94]. There was no substantial difference in bacterial composition after green tea supplementation in normal human subjects, and this could be due to their “optimum” state of energy balance [51]. However, more human trials are needed to confirm this.

Previous studies have shown that obese animals and humans have higher *Firmicutes*/*Bacteroidetes* ratios and higher *Firmicutes* compared with normal-weight individuals, proposing this ratio as a potential biomarker of obesity [95–99]. However, few studies proved that a high-fat diet decreased both bacteria levels [70, 77]. Tea
supplementation increased the *Firmicutes: Bacteroidetes* ratio and *Firmicutes* compared with in the high-fat group alone [70, 77]. A recent human trial showed a higher *Firmicutes:Bacteroidetes* ratio and higher *Firmicutes* in normal-weight subjects after tea supplementation [52].

Meta-analyses failed to observe a clear correlation between the ratios of these two phyla and obesity, suggesting the complexity of how the gut microbiome modulates obesity [100]. Although the gut microbiota could contribute to the development of obesity, the evidence suggesting an association between obesity and alterations of the *Firmicutes: Bacteroidetes* ratio and *Firmicutes* is not convincing [81]. Thus, tea certainly has effects on relative species abundance of gut microbiota, although interpretation of the findings is still lacking [43].

In general, this review showed that low doses of tea might increase gut microbiota diversity in a short period of time, compared with higher tea doses given for a longer period. A longer period of consumption with higher doses diminished the effects observed during a short period of supplementation. This suggests that human gut microbiota are resilient towards longer and higher doses of tea supplementation. Human microbiota are stable upon reaching adulthood, and the composition of gut microbiota remains relatively unaffected by acute perturbations, since its plasticity-like characteristics allows it to return rapidly to its initial composition [101, 102]. This review showed high variability in terms of different types of tea, food matrix, doses and duration of tea supplementation. Each study used a different type of approach, i.e. richness, evenness, relative abundance and β diversity.

5. Conclusions

Tea could increase alpha and beta diversity of gut microbiota in animals, regardless of tea type, forms, dosage and duration of intake. However, little effect was observed in humans due to a higher inter-variation in gut microorganisms between individuals. However, the exact mechanism of how tea affects trillions of microbiota in the gut is still poorly understood. More vigorous studies and trials on tea and gut microbiota are needed to understand the effects. While new evidence is needed, *Camellia sinensis* should be considered as a source of polyphenols in the diet. However, given the differences within and between human and animal studies, there is no specific dose and duration of tea that could be recommended for healthy gut microbiota.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Figure S1: title, Table S1: title, Video S1: title.

Author Contributions: Khairudin, M.A.S. researched and wrote the paper. Mhd Jalil, A.M. and Hussin, N. planned, conceptualized and edited the paper. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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abolism and autophagy in the mammalian colon.


