Chemistry and health beneficial effects of oolong tea and theasinensins

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Abstract

Among six major types of tea (white, green, oolong, yellow, black, and dark teas) from Camellia sinensis, oolong tea, a semi-fermented tea, with its own unique aroma and taste, has become a popular consumable as indicated by the increasing production. Representing the characteristic flavonoids of oolong tea, theasinensins are dimeric flavan-3-ols. Many recent studies have indicated that oolong tea and theasinensins possess several health benefit properties. We consider it significant and necessary to have a comprehensive review in the recent advances of oolong tea. Therefore, the aim of the present review is to provide a new perspective on oolong tea and its characteristic phytochemicals, theasinensins associated with health benefits, molecular action pathway, and chemical mechanism of theasinensins formation from scientific evidences available on the literature. Furthermore, the chemical characterization of the oxidation products and the model oxidation system to the chemical changes of theasinensins are also discussed.

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1. Introduction

Generally, functional foods may be classified into three categories: food to which a component has been added, food in which a component has been modified in nature and/or bioavailability, and conventional food containing naturally occurring bioactive substance [1]. For the later class, tea from Camellia sinensis is one of the most important functional foods and has held the second most popular beverage in consumption among all beverages except water worldwide. Tea contains over 4,000 chemicals and some of which have health promoting properties [2].

Based on different degree of fermentation during tea process, tea can be simplistically divided into three major types: green (unfermented) tea produced from fresh tea leaves and enzymatic oxidation is inhibited using steaming or pan-frying; oolong (partially-fermented) tea made by wilting fresh leaves by sun, then slightly bruising; and black (fully fermented) tea made by crushing tea leaves to release the polyphenol oxidase and peroxidase for fully catalyzing the enzymatic oxidation and polymerization of original tea catechins [3–6]. Many potential health promotion properties associated with these three types of tea consumption have been reported [7,8]. Comparing with
Different Adapted green Types are various and consumed in England, from new technologies and consumer demand increased in the last decades. Among three types of tea, black tea is the most popular tea produced and consumed preferentially in the United State, England, and other Western countries with 78% of global market, followed by 20% of green tea consumed primarily in Asian and Northern African countries, and about 2% of oolong tea consumed mainly in Taiwan, southern China, and most Eastern countries [13,14]. It has been noted that production and consumption of oolong tea worldwide have increased over the past decades. For example, the production of oolong tea in China from 2000 to 2014 had been nearly doubled and increased from $67.6 \times 10^3$ to $254 \times 10^3$ metric tons [15]. Catechin prior to oolong tea is oxidized in the range of 10–80% during processing depending on the demand of customers [9,16]. The taste quality of oolong tea depends on several properties, such as smell of volatile fragrance, taste sensation of sweetness, umami, and intensity of astringency. The differentiation of green tea, black tea and oolong tea, regardless of degree of fermentation, is also dependent on their contents of free amino acids, mainly $\gamma$-glutamyl and several natural amino acids including glutamic acid, asparagine, serine, alanine, leucine, and isoleucine [17].

Major contents of oolong tea infusion are listed in Table 2, which has two categories: monomeric polyphenols and polymeric substances. Oolong tea have been demonstrated to possess various pharmacological activities such as antioxidant activity and anti-atherosclerotic effects [13,14].

### Table 1: Different fermented tea and catechin contents.

<table>
<thead>
<tr>
<th>Types of tea</th>
<th>Fermentation</th>
<th>Total catechins (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pu-erh tea</td>
<td>Microbial fermentation</td>
<td>6.07 ± 0.18</td>
</tr>
<tr>
<td>Iron Buddha tea</td>
<td>Semi-fermentation</td>
<td>7.49 ± 0.22</td>
</tr>
<tr>
<td>Oolong tea</td>
<td>Semi-fermentation</td>
<td>8.05 ± 0.18</td>
</tr>
<tr>
<td>Jasmine tea</td>
<td>Less fermentation (reprocessed from loose green tea scented with fresh Jasmine flower)</td>
<td>12.72 ± 0.70</td>
</tr>
<tr>
<td>Lung Chen tea</td>
<td>Less fermentation</td>
<td>14.57 ± 1.08</td>
</tr>
</tbody>
</table>

Adapted and modified from Sajilata et al. [5].

Green tea having only monomeric catechins, *i.e.* (-)-epicatechin (EC), (-)-epicatechin-3-gallate (ECG), (-)-epigallocatechin (EGC), and (-)-epigallocatechin-3-gallate (EGCG), fully fermented black tea and semi-fermented oolong tea contain a mixture of catechins and their oxidized polymeric substances such as theaflavins and thearubigins [9]. Comparison of catechin contents in different fermented and semi-fermented teas is illustrated in Table 1. Theaflavins (TFs) and thearubigins (TRs), main secondary polyphenols formed during fermentation process by enzymatic oxidation, have been extensively studied on bioactivities and formation mechanism. The orange red or brown color and astringent taste of black tea infusion is attributed to TFs as TRs contribute to rusty color and richness taste [4,10]. There are four major TFs in black tea and oolong tea, that is, theaflavin (TF1), theaflavin-3-gallate (TF2a), theaflavin-3′-gallate (TF2b), and theaflavin-3,3′-digallate (TF3) [6,11,12]. Chemical structures of various types of tea catechins and theaflavins are shown in Fig. 1.

Among three types of tea, black tea is the most popular tea produced and consumed preferentially in the United State, England, and other Western countries with 78% of global market, followed by 20% of green tea consumed primarily in Asian and Northern African countries, and about 2% of oolong tea consumed mainly in Taiwan, southern China, and most Eastern countries [13,14]. It has been noted that production and consumption of oolong tea worldwide have increased over the past decades. For example, the production of oolong tea in China from 2000 to 2014 had been nearly doubled and increased from $67.6 \times 10^3$ to $254 \times 10^3$ metric tons [15]. Catechin prior to oolong tea is oxidized in the range of 10–80% during processing depending on the demand of customers [9,16]. The taste quality of oolong tea depends on several properties, such as smell of volatile fragrance, taste sensation of sweetness, umami, and intensity of astringency. The differentiation of green tea, black tea and oolong tea, regardless of degree of fermentation, is also dependent on their contents of free amino acids, mainly $\gamma$-theanine and several natural amino acids including glutamic acid, asparagine, serine, alanine, leucine, and isoleucine [17].

Major contents of oolong tea infusion are listed in Table 2, which has two categories: monomeric polyphenols and polymeric substances. Oolong tea have been demonstrated to possess various pharmacological activities such as antioxidant activity

### Table 2: Components of oolong tea beverage.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Contents (mg/100 mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catechin</td>
<td>1.65</td>
</tr>
<tr>
<td>Gallatechin</td>
<td>6.68</td>
</tr>
<tr>
<td>Epigallocatechin</td>
<td>16.14</td>
</tr>
<tr>
<td>Epicatechin</td>
<td>5.08</td>
</tr>
<tr>
<td>Catechin gallate</td>
<td>0.6</td>
</tr>
<tr>
<td>Epicatechin gallate</td>
<td>5.73</td>
</tr>
<tr>
<td>Epigallocatechin gallate</td>
<td>25.73</td>
</tr>
<tr>
<td>Allocatechin gallate</td>
<td>1.85</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>2.19</td>
</tr>
<tr>
<td>Caffeine</td>
<td>23.51</td>
</tr>
<tr>
<td>Polymerized</td>
<td>33.65</td>
</tr>
<tr>
<td>Total polyphenols</td>
<td>99.32</td>
</tr>
</tbody>
</table>

Sajilata et al. [5].
by reducing oxidative stress, anti-cancer, anti-obesity, anti-diabetes, preventive effect of atherosclerosis, heart disease, hypertension, anti-allergic effect, and anti-septic effects [18–27]. However, all of the studies were applied to oolong tea extract, not single characteristic oolong tea polyphenols. Until 1984, a new group of polymeric oxidized flavan-3-ols was isolated and identified from oolong tea as theasinensins A, B, C, and later in 1988 for D, E, F and G, have been confirmed from oolong tea by the Japanese scientists Nonaka and Hashimoto [28–33]. As a matter of fact, this group of compounds was formerly discovered by Robert in 1958 [34,35] and was known as bisflavanol A, B, and C, which were formed by coupling of EGC. Based on the literatures listed above, theasinensins were implied as the bioactive flavonoids in oolong tea.

2. Theasinensins – structure and occurrence

Theasinensins are formed from catechin dimerization at their B-rings, i.e. two catechin B-rings are connected through C-C bonds [35,37]. The major tea polyphenol components found in black and oolong teas are shown in Fig. 1. The configuration of theasinensins A, B, and C differ from that of theasinensins D and E in which the biphlenyl bonds of theasinensins A, B, and C, carrying a R-biphenyl configuration, whereas theasinensins F have S-biphenyl bonds. Hashimoto et al. [33] also concluded that theasinensins D and E were characterized as the atropisomers/stereoisomers of theasinensins A and C, respectively. Theasinensin A and D are the dimers of EGC with an R- and an S-biphenyl bond, respectively [37]. Theasinensin B is the dimer of EGCG and EGC and theasinensin C is the dimer of EGC [38].

Theasinensins, mainly exist in black tea and oolong tea [39], are transformed from the unstable intermediate produced by the oxidation of original catechins in tea leaves [28–33]. Among tea catechins found in fresh tea leaves, EGC is the dominant catechin (63%) followed by EGC (25%) [5,40,41]. Therefore, oxidation of EGC and EGCG, two pyrogallol-type catechins, is important during fermentation process. Many studies had been focused on the oxidation of these two catechins to understand the formation of theasinensins. Several studies have been designed to clarify the underlying mechanisms of theasinensin formation in the fermentation process including a model system to mimic catechin oxidation [33,35,37,42,43].

3. Proposed formation mechanism of theasinensin via galloocatechin dimerization

Hashimoto et al. [42] indicated that tea catechins are easily transformed to theasinensins by endogeneous polyphenol oxidase than to theaflavins which are the characteristic flavonoids in black tea. This finding has unequivocally confirmed that theasinensin production involved in the enzymatic oxidative coupling of two pyrogallol rings of EGCG. The pyrogallol rings are also susceptible to be oxidized by chemical agents [44]. There are different pathways toward the formation of theasinensins and theaflavins. The benzotropolone core of TFs is formed from an oxidative condensation reaction between a catechol moiety of EC or ECG and a pyrogallol moiety of EGC or EGCG [42], whereas theasinensin carrying a R-biphenyl bond is generated by a pyrogallol-type B rings of EGCG and EGC.

Once the fresh tea leaves are crushed and kneaded, TFs are formed in the leaves, but theasinensins are not detected in this step [28,33]. The formation of theasinensins is observed in heating the leaves to 80 °C [28,33,35]. Several researchers who tried to investigate theasinensin formation pathway initially focused on characterizing the intermediates and later known that they were heat and chemical susceptible intermediates. The structure elucidation from derivatization of the unstable intermediates has concluded that they are quinone dimers of EGC and EGCG produced by these two catechin quinone monomers. To date, one of the intermediates has been successfully synthesized by an enzymatic oxidation of EGCG and named dehydrotheasinensin A with a pale yellow color [37,43]. In the heating and drying process of the tea leaves at the final stage of black and oolong tea manufacturing, dehydrotheasinensin A undergoes redox dismutation to generate theasinensin A and its atropisomer [37,43]. The production of unstable dehydrotheasinensins from the oxidation of pyrogallol-type B-ring catechins is the most important reaction in theasinensin formation. Takana et al. [38] have utilized an in vitro experiment to investigate the chemical mechanism of theasinensin formation and the proposed pathway is also given in Fig. 2.

More than a decade ago, the oxidized EGCG and EGC as theasinensin quinones were trapped for the first time with o-phenylenediamine, yielding five phenazine derivatives of catechin dimer quinones [35]. The result was also the first evidence supporting the formation and accumulation of catechin dimer quinones in tea fermentation process and proving that the formation mechanism of theasinensin A is not simply produced by enzymatic oxidation of EGCG, the main catechin in fresh tea leaves. These theasinensin quinones are unstable and decomposed readily in particular the process of extract solution concentration by rotary evaporator [35]. Later, Tanaka’s team also suggested that the addition of 0.1% trifluoroacetic acid (TFA) in the mobile phase increased the stability of theasinensin quinones [37].

Tanaka and his team were also focused on the chemical examination of theasinensin production using in vitro experiments to mimic black tea production process in particular on the fermentation step. EGCG and enzymes from homogeneous pear were applied at room temperature for 2 h. The study elucidated the structure of dehydrotheasinensin A and mechanism of theasinensin formation, and successfully obtained dehydrotheasinensin A, a theasinensin precursor from enzymatic oxidation of EGCG. The results also revealed that dehydrotheasinensin A produced from coupling of EGCG quinone monomers is equivalent to a hydrated ortho-quinone of theasinensin A, which is readily converted to theasinensin A and D by oxidation reduction dismutation. Previous studies from Takana et al. [35,37] suggested that several chemical reactions involved in EGCG oxidation subsequently produced theasinensins (A and D).

EGCG was initially oxidized to the ortho-quinone, and subsequently an unstable dimer, theasinensin quinone was formed after stereoselective dimerization (Fig. 2). This unique quinone
was equivalent to dehydrotheasinensin A, generated by both enzymatic and non-enzymatic reaction [28,33,35,37,43,45,46]. To further understand the chemical mechanism of theasinensin formation, many studies had concentrated on the enzymatic oxidation of catechins such as EGC oxidation. However, several studies also focused on the non-enzymatic oxidation of catechins.

The non-enzymatic oxidation of EGC providing theasinensin quinones can be developed from not only chemical oxidation with either potassium ferricyanide or copper salts, but also autoxidation in a phosphate buffer (pH 7.4 or 6.8) in which the oxidized product is unstable even at a neutral pH [37]. Therefore, it can be implied that the production of theasinensin A and D derived from dehydrotheasinensin A during black tea and oolong tea manufacturing may possibly be a non-enzymatic process which occurs spontaneously when tea leaves are heated and dried. Shii further showed that non-enzymatic oxidation of EGC by copper salts with the optimum pH of 4–5 at room temperature yielded dehydrotheasinensin A and the product increased with the elevated temperature [43]. Initial oxidation reaction was carried out at room temperature and elevated temperature increased byproduct generation. The difference between enzymatic oxidation in tea leaves and in vitro chemical oxidation by copper salt is that catechol-type catechins prefer to enzyme catalyzed oxidation, such as EC and ECG, whereas pyrogallol-type catechins, such as EGC and EGCG, are less reactive [43,45]. By enzymatic oxidation (banana homogenate) of catechin, EGCG was oxidized to the corresponding catechin quinone but the reaction was accelerated in presence of ECG. The oxidation of ECG was much faster than EGC due to its higher redox potential than pyrogallol-type catechin (EGCG, EGC) [35,43]. Technically, the catechol-type catechins were not chemically oxidized by copper salt. Therefore, the chemical oxidation of catechins does not yield theaflavins which are generated from the oxidative couplings between the quinones produced from catechol-type and pyrogallol-type catechins [47–49].

Enzymatic oxidation of EGC generates a new quinone dimer with a hydrated cyclohexenetrione structure, which may be equivalent to dehydrotheasinensin C whereas theasinensins C and E were produced through oxidation–reduction dismutation [39]. In addition, the enzymes isolated from pear homogenates catalyzed the oxidation of EGC at the pyrogallol B-ring, yielding predominantly unstable quinone products known as dehydrotheasinensin C trapped with o-phenylenediamine [45]. Non-enzymatic coupling reaction of dehydrotheasinensin C was subsequently occurred to give theasinensins A and C [37,38,45,46].

From commercial back tea, an interesting derivative of theasinensin, N-ethylpyrrolidinonyl theasinensin has been isolated and identified [44,50]. Tea leaves contain an amino acid named L-theanine (N\(^\beta\)-ethyl-L-glutamine) accounting for over 50% of the total amino acids. Consequently, the catechin quinones generated from the beginning of tea process maybe react with L-theanine to generate theanine Strecker aldehyde [51,52] as demonstrated in Fig. 3. The Strecker aldehyde of theanine subsequently reacts with theasinensin to yield N-ethylpyrrolidinonyl theasinensin.

In addition, the reduction of dehydrotheasinensin A to yield theasinensin derived from several paths. The formation of theasinensins through several chemical reactions was involved in EGCG oxidation [35,37,45]. Dehydrotheasinensin A could be reduced by the reductants such as ascorbic acid or sulfur containing compounds at room temperature and subsequently forming theasinensin A [37]. It is also known that reduction of dehydrotheasinensin A with 2-mercaptoethanol afforded theasinensin A only. Stereoselective formation of dehydrotheasinensin A from EGCG created a chiral center at the benzylic position of the pyrogallol ring [46,53]. Unstable dehydrotheasinensin A was rapidly degraded by heating alone or heating with ascorbic acid, higher
pH, and theasinensin A subsequently was formed [37,43]. Even so, the reducing agent such as 2-mercaptoethanol had lower yield of theasinensin A compared to ascorbic acid [43]. On the other hand, the reduction of dehydrotheasinensin A occurred at 80 °C and thermodynamically underwent the oxidation–reduction dismutation to yield theasinensin A. Theasinensin D also was produced as it is probably isomerized at an elevated temperature during heating and drying in black tea manufacturing. Furthermore, theasinensin quinones were gradually decomposed to a mixture of theasinensin A and D in a pH 6.8 phosphate buffer at 20 °C [37]. Taken together, it is clear that the products from enzymatic oxidation of tea catechins both in oolong and black tea process can be classified into two major oxidation routes [4,35,38,42,46]: the condensation with coexisting epicatechin forming theaflavins [47,48] and oxidative dimerization of two gallo moiety and reduction yielding epigallocatechin dimers, such as theasinensins and oolongtheaamins [28,33].

4. Potential bioactive compounds

Evidence from experimental and clinical studies has indicated that tea exerts antioxidative, anti-inflammatory, cancer prevention and vasodilating effects among others [54]. Epidemiological studies, both in vitro and in vivo, have indicated that tea consumption is positively associated with reduced risk of chronic diseases, causing 60% global death such as coronary heart disease, stroke and cancer [54–58]. One of the health benefits of tea polyphenols is generally attributed to its potent antioxidant property. Act as free radical scavengers, tea polyphenols may remove endogenously superoxide, peroxyl, and hydroxyl radicals [22,59,60]. Tea polyphenols and their metabolites also possess antibacterial properties against pathogenic bacteria such as *Clostridium perfringens*, *Clostridium difficile*, *Escherichia Coli*, *Salmonella*, and *Pseudomonas* and enhance probiotics such as *Bifidobacterium* and *Lactobacillus* species, which improve the intestinal microbial balance [61,62].

The study on bioactivities of oolong tea and its characteristic compounds, theasinensins on health promotion property in the literature is still limited compared to that of black and green teas. Several studies have revealed that oolong tea and theasinensins have biological activities such as antioxidative effects against lipid peroxidation, anti-inflammatory activity, antibacterial properties and anti-obesity. Herein, we summarize the health benefits of oolong tea and theasinensins in Tables 3 and 4.

4.1. Antioxidant activities of oolong tea and theasinensins

The FRAP (ferric reducing/antioxidant power) assay has tested that antioxidant value of oolong tea ranged between 233 and 532 μmol/g [63]. In an *in vivo* study, modest transient increase in human plasma antioxidant capacity was noticed upon oolong tea consumption [22]. It is observed that oolong tea reduced oxidative stress, especially oxidative DNA damage [22]. Furthermore, human studies on athletes showed that oolong tea ingestion significantly reduced plasma malondialdehyde levels in rest and post-exhaustive exercise athletes, as well as...
resting levels of superoxide dismutase activity, suggesting that the decrease of oxidative stress is resulted from reduction of the lipid peroxidation level and its free radical scavenging activity [25]. Hashimoto reported that the antioxidant activity evaluated by the 5-day lipid peroxidation of theasinensins A–E were ranged from 9 to 13% compared to 3 and 17% for BHA and alpha-tocopherol, respectively. The result suggests that the inhibitory activity on lipid oxidation of theasinensins A–E were lower than BHA (the synthetic antioxidant), but higher than alpha-tocopherol [64]. Among theasinensin isomers, theasinensin C has the highest ability against lipid oxidation inhibition. Until now, there is no data reported concerning on bio-antioxidative effect of oolong tea or theasinensin on the antioxidant defense systems.

### 4.2. Anti-inflammatory effect

Previous studies have indicated that ethanol extract of oolong tea was profoundly increased adiponectin gene expression in epididymal fat, consistent with an anti-inflammatory effect, and angiogenesis during adipose tissue expansion [65]. The increase of blood vessel formation in adipose tissue contributes to their anti-inflammatory effects by maintaining adipocyte perfusion [65]. This finding is consistent with the pro-angiogenic activity of oolong tea ethanol extract during adipose tissue expansion mediating protective effects on metabolism and inflammation, although this finding is in contrast to the traditional concept that inhibition of angiogenesis results in weight loss [65,66]. In addition, ethanol extract of oolong tea decreased the concentration of monocyte chemoattractant protein-1 (MCP-1) in serum. The reduction of plasma MCP-1 also explains the anti-inflammatory effect of oolong tea polyphenol since MCP-1 is a small cytokine recruiting monocytes, memory T cells, and dendritic cells to the site of injury [67]. The anti-inflammatory activity of oolong tea might be due to flavonoids such as catechins, theaflavins and theasinensins [68]. There are some reports on the anti-inflammatory properties of theasinensins. Results from the first molecular basis of the anti-inflammatory properties of oolong tea theasinensins showed that theasinensin A and D exhibited better activities in reducing LSP-stimulated COX-2 and PGE2 production than theasinensins B, C and E. Structure analysis of theasinensins A and D with two galloyl moieties and theasinensins C and E without galloyl moieties, showed that the galloyl moiety played an important role on anti-inflammatory effects of oolong tea theasinensins. Molecular mechanisms of anti-inflammatory property of oolong tea theasinensins demonstrated that the down-regulation of TAK1-mediated NF-κB and MAPK signaling pathway might be involved in the inhibition of COX-2 expression by theasinensin A [68]. Recently, in clarifying the molecular mechanism of anti-inflammatory effects of
<table>
<thead>
<tr>
<th>Bioactivity</th>
<th>Experiment model</th>
<th>Compound tested/control</th>
<th>Mechanism/biomarker</th>
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<td>In vivo</td>
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<td>Antioxidant activity</td>
<td>Lipoxygenase inhibition activity</td>
<td>Fraction compound from oolong tea</td>
<td>Antioxidant power</td>
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<td>FRAP assay</td>
<td>Tea infusion (green, oolong and black tea)</td>
<td>Reducing oxidative DNA damage</td>
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<td>Human plasma</td>
<td>Consumption oolong tea infusion</td>
<td>Normalizing the cholesterol profile, reducing lipid peroxidation level, and superoxide dismutase activity</td>
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<td>Athletes before and after exhaustive exercise</td>
<td>Daily consumption of oolong infusion for 30 days</td>
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<td>Anti-inflammation</td>
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<td>Reducing MCP-1 plasma concentration</td>
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<td></td>
<td>Mice</td>
<td>Crude ethanol extract of oolong tea</td>
<td>Reducing MCP-1 gene expression</td>
<td>[65]</td>
</tr>
<tr>
<td>Anti-obesity</td>
<td>High-fat diet-induced obese mice</td>
<td>Oolong tea</td>
<td>Fat cells and a cell-free system consisting of lipid droplets and hormone-sensitive lipase</td>
<td>[72]</td>
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<td>Rats</td>
<td>Oolong, black, pu-erh, and green teas leaves</td>
<td>Reducing plasma lipid</td>
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<td>Salmonella/microsome reverse mutation assay (Salmonella typhimurium TA98 and TA100)</td>
<td>Tea water extract (green, oolong, pouchong, and black teas)</td>
<td>Anti-mutagenicity</td>
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<td>Salmonella/microsome reverse mutation assay (Salmonella typhimurium TA98 and TA100)</td>
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<td>Male F344 rats induced by diethyl/nitrosamine (rat model is in vivo study)</td>
<td>Tea extract (black and oolong tea/tea catechins)</td>
<td>Suppressing hepatocarcinogenesis</td>
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<td>Salmonella/microsome reverse mutation assay (Salmonella typhimurium TA100, TA98 and TA97)</td>
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<td>Preventing proliferation and invasion of AH109A cells cells</td>
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<td>Male Donryu rats</td>
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<td>Inducing apoptosis, and cell cycle arrest</td>
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<td>Human stomach cancer KATO III cells</td>
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<td>Salmonella/microsome reverse mutation assay (Salmonella typhimurium TA1535/pSK 1002)</td>
<td>Tea methanol extract (green, pouchong, oolong and black tea)</td>
<td>Anti-genotoxic abilities (suppressive effects against umu gene expression)</td>
<td>[80]</td>
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<td>Hypoglycemic effect</td>
<td>Adult female frogs (Xenopus laevis)</td>
<td>Tea extract by hot normal frog Ringer Solution (green, oolong, and black tea)</td>
<td>Inhibition of SGLT1 Response</td>
<td>[86]</td>
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<tr>
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<td>Rat epididymal adipocytes</td>
<td>Tea infusion (green, oolong, and black tea)</td>
<td>Enhancing insulin-activity</td>
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</tbody>
</table>
theasinensin A, gene expression profiling in macrophage-like cells treated with theasinensin A through a genome-wide DNA microarray were used to detect the changes of 22,050 genes involving inflammatory and immune response. The changes of 1382 genes suggested that theasinensin A has exerted anti-inflammatory effects by regulating the relevant expression networks of chemokines, interleukins, and interferons [69]. Recently, an in vitro study on anti-inflammatory activity of theasinensin A by LPS-activated macrophages indicated that the levels of pro-inflammatory mediators including inducible nitric oxide synthase (iNOS), nitric oxide (NO), interleukin-12 (IL-12) (p70), tumor necrosis factor alpha (TNF-α), and MCP-1 were significantly reduced by theasinensin A. Cellular signaling pathway of this study uncovers that theasinensin A downregulated MAPK/ERK kinase (MEK)-extracellular signal-regulated kinase (ERK) signaling through directly binding to MEK-ERK for the inhibitory action. The in vivo study also demonstrates that the theasinensin A suppressed the production of IL-12 (p70), TNF-α, and MCP-1 and attenuated mouse paw edema induced by LPS [70].

4.3. Anti-obesity

Nowadays obesity has emerged as a major health concerning problem and a risk factor of metabolic disorders. Functional foods affecting energy metabolism and fat partitioning may be helpful adjuncts to a dietary approach to weight control. The mechanism of anti-obesity effects of tea catechins, especially EGCG and theaflavins, appear to be related to modulation of energy balance, endocrine systems, food intake, lipid and carbohydrate metabolism, and activities of different cell types including fat, liver, muscle and β-pancreatic cells [7,27]. Tea catechins inhibit lipogenesis through down-regulation of gene expression of fatty acid synthesizes in the nucleus, resulting in the down-regulation of EGFR/P13K/Akt/Sp1 signal transduction pathways, and stimulation of cell energy expenditure in the mitochondria [27,69]. One of the mechanisms on fat reduction by oolong tea is the inhibition of pancreatic lipase activity. A mouse study suggested that inhibition of digestive lipase activity can significantly affect dietary lipid absorption and increase lipid excretion into the feces [71]. Another study was

Table 4 (Continued)

<table>
<thead>
<tr>
<th>Bioactivity</th>
<th>Experiment model</th>
<th>Compound tested/control</th>
<th>Mechanism/biomarker</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevention of Heart Disease</td>
<td></td>
<td>10 men and 10 women (average age 61.2 years, duration of diabetes 4.8 years) Male Sprague-Dawley rats</td>
<td>Decreasing plasma glucose and fructosamine</td>
<td>[88]</td>
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<td></td>
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<td>Male Sprague-Dawley rats</td>
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<td></td>
<td></td>
<td>1500 ml of oolong tea per day (tea bags) for 10 weeks</td>
<td>Decreasing serum and liver lipids</td>
<td>[101]</td>
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<tr>
<td></td>
<td></td>
<td>Tea solutions for 9 weeks (green, Jasmine, Iron Buddha, oolong and Pu erh tea)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Water plus caffeine and oolong tea</td>
<td>Increasing EE and fat oxidation</td>
<td>[100]</td>
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<tr>
<td></td>
<td></td>
<td>Medication during their additional oolong tea for 1 month</td>
<td>Decreasing plasma adiponectin, glucose and hemoglobin A1c levels</td>
<td>[97]</td>
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<tr>
<td></td>
<td></td>
<td>12 patients with previous myocardial infarction and 10 patients with stable angina pectoris</td>
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<td>12 healthy men</td>
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<tr>
<td></td>
<td></td>
<td>12 patients with previous myocardial infarction and 10 patients with stable angina pectoris</td>
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<td></td>
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<tr>
<td></td>
<td></td>
<td>12 healthy university students, 3 males and 9 females</td>
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<tr>
<td></td>
<td></td>
<td>12 healthy Japanese females</td>
<td></td>
<td></td>
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<tr>
<td>Anti-microbial effect</td>
<td></td>
<td>711 men and 796 women without hypertensive history</td>
<td>Epidemiology study (tea consumption)</td>
<td>[94]</td>
</tr>
<tr>
<td>Streptococcus sobrinus 6715</td>
<td>Pathogen</td>
<td>711 men and 796 women without hypertensive history</td>
<td>Oolong tea extract and green tea</td>
<td>[99]</td>
</tr>
<tr>
<td>Bacillus subtilis, Escherichia coli, Proteus vulgaris, Pseudomonas fluorescens, Salmonella sp. and Staphylococcus aureus</td>
<td></td>
<td>711 men and 796 women without hypertensive history</td>
<td></td>
<td>[105]</td>
</tr>
<tr>
<td>S. mutans MT8148R and Streptococcus sobrinus 6715</td>
<td></td>
<td>Methanol extract of oolong tea</td>
<td>Antibacterial activity</td>
<td>[106]</td>
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<td>Dry tea extract (green, oolong, and black tea)</td>
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<td>O aqueous tea extract (green and oolong tea) catechin, theasinensin A, theaflavin</td>
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<td>O aqueous tea extract (green and oolong tea)</td>
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Superoxide dismutase (SOD); minimum inhibitory concentration (MIC); maximum non-inhibitory concentration (MNIC); energy expenditure (EE); glucosyl transferase (GTase).
also demonstrated that the oolong tea water extract enhanced noradrenaline-induced lipolysis and inhibited pancreatic lipase activity, resulting in anti-obesity effects. This might be partially due to the effects of caffeine or some other bioactive compounds in oolong tea [72]. The homobiflavans A and B, typical compounds in oolong tea, exhibit more potent inhibitory activities against pancreatic lipase with IC50 values of 0.048, and 0.108 μmol/L, respectively, than EGC with IC50 values of 0.349 μmol/L. The data hinted that galloyl moieties in the molecule were crucial for pancreatic lipase inhibition [73]. Supplementation with oolong tea leaves reduced body weights and plasma triacylglycerol, cholesterol and LDL-cholesterol of rats. Pu-erh tea and oolong tea can significantly decrease the levels of triacylglycerol more significantly than green tea and black tea. In addition, superoxide dismutase activity (SOD) was increased and the relative weight ratios of liver to epididymal adipose tissue reduced by oolong tea. This study also suggested that oolong tea was more effective on their growth suppressive and hypolipidemic effects as compared to green tea [74]. Scientific evidence has revealed the role of MCP-1 gene expression in the etiologies of obesity- and diabetes-related diseases [75]. Plasma concentration of MCP-1 is positively associated with obesity [67]. A recent study shows that the ethanol extract of oolong tea polyphenol caused weight loss in mice fed with an high-fat diet, and decreased plasma MCP-1 protein as well as its gene expression in mesenteric fat and epididymal fat [65].

4.4. Anti-cancer

Among the biological activities of tea polyphenols, the cancer-chemopreventive effects in various animal models have been intensively investigated [76]. Many studies have reported that the anti-cancer effect of oolong tea mostly focused on catechins. However, some studies paid much attention to evaluate the anti-cancer effects of theasensins. It is well known that inflammation plays a key role in the initiation and/or progression of multiple types of cancers, including liver, bladder and gastric cancers by inducing oxidative stress and promoting cell growth [77]. As mentioned above, oolong tea extract exhibits higher anti-inflammatory activity than green tea and black tea extracts [65]. The anti-cancer activity of oolong tea may be originated from its antioxidant and anti-inflammatory activities.

The mutagenic effects of carcinogens such as heterocyclic amines are reduced by oolong tea extracts. In Salmonella reverse mutation assay (Ames test), Yen and Chen [18] indicated that the greater anti-mutagenic effect was found in oolong tea than in green tea and black tea and some anti-mutagenic substances might be formed during manufacturing processes of tea. Ames test with bacterium Salmonella typhimurium has confirmed anti-mutagenic activities of EGC, GC, and caffeine from green tea [78]. Furthermore, oolong tea extract remarkably inhibited the mutagenicity of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), 3-amino-1,4-dimethyl-5H-pyrido-(4,3-b)indole (Trp-P-1), 2-amino-6-methyl-dipyrido(1,2-a:3′,2′-d)imidazole (Glu-P-1), benzo[a]pyrene (B[a]P) and aflatoxin B, (AFB,) and the inhibitory effect was associated with the contents of catechins and ascorbic acid [79]. Oolong tea extract possessed the highest anti-mutagenic activity against several mutagens, including four nitroarenes, two nitro compounds and one alkylating agent (1-nitropyrene (1-NP), 2-nitropyrene (2-NF), 3-nitropyrene (3-NF) and 2,4-dinitrophenol (DNP) among the different teas [79]. In addition, oolong tea extracts have a chemopreventive action against hepatocarcinogenesis. Different concentrations of oolong tea extract (0.05 or 0.1%) significantly decreased the number and area of diethyl-nitrosamine- and phenobarbital-induced preneoplastic glutathione S-transferase placental form-positive foci in the liver of rats [19]. Oolong tea extracts also induced the formation of reactive oxygen species (ROS) and induced cytochromes P450 1A1, 1A2, and 2B1, and glucuronosyl transferase, leading to glucuronide, which is an important mechanism in biological detoxification system [76]. Therefore, one of the anti-carcinogenic mechanisms of oolong tea may be involved in regulation of catalytic activities of the P450 enzymes and glucuronosyl transferase [77,76].

Besides the anti-inflammatory effects, anti-genotoxic and anti-mutagenic properties of oolong tea also contribute to its chemopreventive effect. Zhang et al. [21,81] suggested that the chemopreventive mechanism of oolong tea might be attributed to its bioactivities against the invasion and proliferation of cancer cells (AH109A) through the loss of cell viability, apoptosis, and cell cycle arrest at the G1 phase in a rat hepatoma cell line (AH109A) and murine B16 melanoma cells. Furthermore, the oolong tea extract inhibited the growth of human stomach cancer KATO III cells by the induction of apoptosis [82]. Saeki et al. [83] has confirmed that a pyrogallol type structure in the B-ring of catechin induced apoptosis of cancer cells compared with the catechins without a pyrogallol-type.

In 1999, an in vitro study showed that comparing ECG and EGC, theasensin D and theaflavin-3,3′-digallate exhibited a weak invasion inhibitory effect determined by the suppression of the gelatin degradation mediated through matrix metalloproteinase (MMP) [84]. However, using human histolytic lymphoma (U937) cell line and acute T cell leukemia (Jurkat) cell line, the study demonstrates that theasensin A from oolong tea induced the process of cell death through the release of cytochrome c and activation of caspase-9 and caspase-3 [85]. Their results also suggested that a linear and specific activation cascade between caspase-9 and caspase-3 in response to cytochrome c released from mitochondrial and apoptosis by activation of the caspases, leading to the process of cell death [85].

4.5. Hypoglycemic effect

Hyperglycemia is the major cause of diabetic angiopathy. The Na⁺-dependent glucose cotransporter (SGLT1) in jejunal transports glucose into epithelial cells. The inhibition of glucose uptake via SGLT1 in the small intestine may prevent hyperglycemia [86]. Aqueous extracts of green, oolong, and black tea failed to exhibit effects on SGLT1 response compared with tea catechins. Black, green, and oolong teas as tea beverages had
shown to increase insulin activity by a minimum of 15-fold in an epididymal fat cell assay [87]. Oolong tea extract can reduce plasma glucose and have a complicated impact on antioxidant systems in diabetic rats [7,87]. Oolong tea is an effective adjunct to oral hypoglycemic agents in the treatment of type 2 diabetes. An in vivo study with respect to type 2 diabetes subjects indicated that oolong tea remarkably reduced concentrations of plasma glucose from 229 to 162.2 mg/dL and fructosamine (from 409.9 to 323.3 μmol/L) [88]. This study also strongly supports the concept of combination therapy because the ingestion of oral anti-hyperglycemic agents and oolong tea simultaneously was more effective in lowering plasma glucose than taking the drugs alone [88].

Theasinensins might play a key role in anti-hyperglycemic activity of oolong tea. Theasinensin A induces anti-hyperglycemic responses in diabetic mice and shows hypotriacylglycerolemic effect in rats by suppressing intestinal fat absorption [89]. Miyata indicated that feeding male KK-Ay mice with diets containing 0.1% theasinensin A for 6 weeks reduced serum glucose levels by greater than 30% and feeding rats with diets containing 0.2% theasinensin A for 4 weeks had higher fecal fat excretion and 33% lower hepatic triacylglycerol without the effect on hepatic fatty acid synthase activity [10]. High fecal excretion of fatty acid is associated with the inhibition of pancreatic lipase by theasinensin A [89]. The result suggested that it might be due to suppression of postprandial hypertriacylglycerolemia, theasinensin A inhibited glucose production in the intestine through suppression of α-glucosidase activity. Theasinensin A inhibited α-glucosidase (AGH) activity (IC50 of 142 μmol/L for maltase and 286 μmol/L for sucrase) evaluated by an immobilized AGH assay system and thus provides a substantially useful prediction of the in vivo suppression of glucose absorption [89].

Qiu et al. [90] proposed the mechanism of anti-hyperglycemic activity of theasinensins A and B using rat skeletal muscle cells (L6 myotubes). Theasinensins A and B were found to promote GLUT4 translocation to the plasma membrane in L6 myotubes through the CaM KK/AMPK signaling pathway, but not through the PI3K/Akt pathway. Moreover, theasinensin A is more effective in stimulating 2-NBDG (2-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxyglucose; a fluorescent glucose analog) uptake than theasinensin B, which is as potent as EGCG, suggesting that the number of galloyl moieties may be associated with the promotion of 2-NBDG uptake in cells [90].

GLUT4 plays a pivotal role in regulating insulin-stimulated glucose transport predominantly in muscle of skeleton, cardiac and adipose tissue [91]. Glucose uptake by muscle and fat cells is regulated by modulating the number of GLUT4 on cell surface. Promoting glucose uptake or improving insulin resistance by increasing GLUT4 translocation to the plasma membrane via the AMP-activated protein kinase (AMPK) pathway raises the possibility to prevent hyperglycemia. The effects of theasinensins A and B on regulation of GLUT4 are differs from that of EGCG that involved in stimulating the phosphatidylinositol 3-kinase (PI3K)/Akt pathway in skeletal muscle to improve insulin sensitivity [90].

4.6. Prevention of atherosclerosis, heart disease, and hypertension

High blood pressure affects millions of people globally [92] and it is associated with atherosclerosis and plaque build-up in the arteries, both of which are considered as main cardiovascular risk factors [93]. The prevention of atherosclerosis can also reduce the risk of developing hypertension and heart disease. An epidemiology study has shown that people with habitual and moderate oolong tea consumption, such as 120 ml/day or more for a year period, significantly reduced the risk of developing hypertension in Taiwan [94].

Not only the level but also the particle size of low-density lipoprotein (LDL) is the major risk factor in the early development of coronary heart disease (CAD) [95,96]. Small particle size of LDL is considered as a great risk factor for CAD [97]. Oolong tea increased plasma adiponectin levels and LDL particle size in CAD patients and decreased hemoglobin A1c (HbA1c). Therefore, oolong tea may have beneficial effects on the progression of atherosclerosis in patients with CAD. As mentioned earlier, oolong tea decreased levels of triglyceride in Sprague-Dawley rats and lowered the relative weight ratios of liver to epididymal adipose tissues [74]. The result also showed that oolong tea was more effective in suppressing the growth of adipose tissues as compared to green tea [74]. In addition, oolong tea may inhibit the oxidized-LDL cholesterol, which is a risk factor for atherosclerosis and heart disease, and reduced the formation of 8-hydroxydeoxyguanosine, a marker of oxidative DNA damage [76].

There is an affiliation between the ingestion of dietary lipid and some diseases such as obesity and cardiovascular diseases. Hence reduction of lipid intake is a logical nutritional intervention strategy. Moreover, the more energy expenditure means less accumulation of lipid, which can decrease the incidence of atherosclerosis and heart disease. To absorb dietary lipids, the lipid digestion by pancreatic lipase is a key step. Inhibition of digestive lipase activity significantly affects dietary lipid absorption, leading to increase lipid excretion into the feces [71]. Thus, it is a possible strategy responsible for the prevention of atherosclerosis and heart disease. Polyphenol-enriched oolong tea extract increased lipid excretion into feces in subjects with high fat diet, and polymerized-polyphenol extract from oolong tea appears to increase cholesterol excretion into feces [98]. Furthermore, the consumption of oolong tea in healthy women leads to the increase of energy expenditure (EE) and this effect is higher than that of green tea [99]. The same trend of the effect of oolong tea on energy expenditure was reported in Japanese subjects [99] and the result showed that oolong tea stimulates fat oxidation. Therefore, the consumption of oolong tea increased metabolic rate and fat oxidation, potential beneficial effect on an individual’s ability to maintain a lower body fat content, associated with lower risk of atherosclerosis and hypertension [100]. Oolong tea decreased atherogenic index and increased HDL-total cholesterol ratio in hypercholesterolemia rats [101]. However, there is still no report of the bioactivity of theasinensins on prevention of atherosclerosis and heart disease in the literature.
4.7. Antiseptic effects

Oxacillin (methicillin)-resistant *Staphylococcus aureus* (MRSA) caused infection is a problem in health care institutions in the United States and worldwide, especially for intensive care unit patients [102]. This type of bacteria is resistant to a number of widely used antibiotics, so MRSA infections can be more difficult to cure than other bacterial infections. Aqueous extract of oolong tea inhibits various pathogens including *Staphylococcus aureus* (a methicillin-resistant strain). A previous study indicated that theasinensin A has the maximum non-inhibitory concentration at 130–180 mg/mL. Hatano et al. [103] found that theasinensin A suppressed the oxacillin resistance of MRSA and the minimum inhibition concentration (MICs) of oxacillin decreased from 64 to 4 μg/mL. It decreased the MICs of other β-lactam including penicillin G, ampicillin, and streptomycin, antibiotics for MRSA strain [104].

Pathogenesis is closely associated with the ability to synthesize water-insoluble glucans from sucrose by glucosyltransferases (GTases) and to release acids from various fermentable sugars [105]. Oolong tea extract can decrease the cellular surface hydrophobicity of almost all the oral streptococcus and inhibit bacterial adherence to the tooth surfaces, as well as reduce the growth rate and the rate of acid production of mutant streptococci [104]. Oolong tea extract and its purified polymeric polyphenols identified as dehydro-dicatechin A exhibited the inhibition of glucosyltransferase (GTase) of mutants streptococci, *Streptococcus sobrinus* 6715. As the degree of polymerization of catechin increased, GTase was inhibited more effectively [105]. Furthermore, antibacterial activity of oolong tea extract on oral streptococci, including *Streptococcus mutans* and *S. sobrinus* has been confirmed by Sasaki’s study who reported that the oolong tea extract have antibacterial activity against all of the oral streptococci examined, with the highest activity against *S. mutans* MT8148R in which the activity was attributed to a monomeric polyphenol-rich fraction. The results also suggested that the antibacterial activity of oolong tea extract was resulted from a synergistic effect of monomeric polyphenols, which can easily bind to proteins [106]. Besides, theasinensin A weakening virus has been reported. For example, herpes simplex virus (HSV), both HSV-1 and HSV-2, is the leading cause of genital ulcers in the developed world [107]. Theasinensin A caused aggregation of HSV-1 glycoprotein B (gB) and the effect is faster than EGCG suggesting that dimers may inhibit the function of viral proteins. This effect is similar to EGCG, on herpes simplex virus (HSV) infectivity. As microbicidal agents against HSV, theasinensin A appears to have excellent potential at acidic and neutral pHs [108].

5. Bioavailability

Theasinensins have been reported to have many biological properties closely associated with the anti-hyperglycemic, anti-obesity, anticancer, anti-inflammatory, antibacterial activity. Usually, biological properties of polyphenols are associated with their bioavailability [109]. Tea polyphenols are biologically regarded as xenobiotics that will be extensively metabolized for elimination from the body [110]. Metabolism of tea polyphenols can be affected by interaction with other dietary ingredients, solubility, molecular transformations, different cellular transporters and the action of gut microbiota, interindividual variations [110]. The Caco-2 cell monolayers, derived from human colon carcinoma, have been widely used as an *in vitro* intestinal absorption model for studying permeability and transport of drugs [111,112]. It represents morphologically the enterocytes of the small intestine and exhibits brush-border characteristics at the apical side [112].

Qiu et al. [112] illustrated the *in vivo* and *in vitro* absorption of theasinensins A and B and evaluated their transport pathway across intestinal membrane. The rat study showed that a single oral administration of theasinensins demonstrated the intact absorption of theasinensins into the blood system, which was estimated to be a greater than 10-fold lower absorption amount than EGCG. The *in vitro* absorption study indicated that theasinensins can be transported across Caco-2 cell monolayers, while their permeability coefficients were also >10-fold lower than those of EGCG and EGC. In addition, theasinensins were transported across Caco-2 cells in a tight junction (TJ) paracellular diffusion pathway which is the same route as EGCG [112].

6. Future studies of theasinensins

We have known that the concentration of theasinensin A is higher than theasinensin D in black tea [44]. Hashimoto et al. [42] also found that the concentration of theasinensin A is higher than theasinensin B in fermented tea leaves. The average contents of theasinensins in green tea and oolong tea are 0.05% and 0.65%, respectively [36,113]. Data on the content of theasinensins in black or oolong tea are still scarce. Comparison of each isoform of theasinensins between black tea and oolong tea is necessary. Many work are needed to clarify pharmacokinetics and bioactivity of theasinensins both *in vivo* and *in vitro* model. Despite there are studies reporting the potential health benefits of theasinensins, the potential biological activities of their metabolites (conjugates or microbial) is poorly understood. Finally, it is also important to highlight that no studies regarding the mechanism of theasinensins (A–E) on obesity and other bioactivity such as hypertension and atherosclerosis associated with heart disease prevention.

7. Conclusion

In summary, oolong tea and its characteristic compounds theasinensins have been reviewed for chemical formation mechanism of theasinensins and their corresponding biological property and potential action pathway, specifically, the bioactivity of oolong tea and theasinensins and their structural information. These studies clearly indicated the various bioactivities on health benefit of oolong tea coming from theasinensins. Regardless of the polymeric nature of oolong tea extract, the results shown in the published literatures implicated a relationship between oolong tea and theasinensins and their biological properties. A better knowledge of bioavailability of theasinensins is required. It is foreseeable that theasinensins
may become a promising class of health products against the development of many diseases.

Conflict of interest

The authors have no conflicts of interest to declare.

Acknowledgments

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References

[43] T. Shii, M. Miyamoto, Y. Matsu, et al., Biomimetic one-pot preparation of a black tea polyphenol theasinensins A from epigallocatechin gallate


