

Beneficial Effects of Green Tea Catechin on Veterinary Sciences and Bacterial Infections

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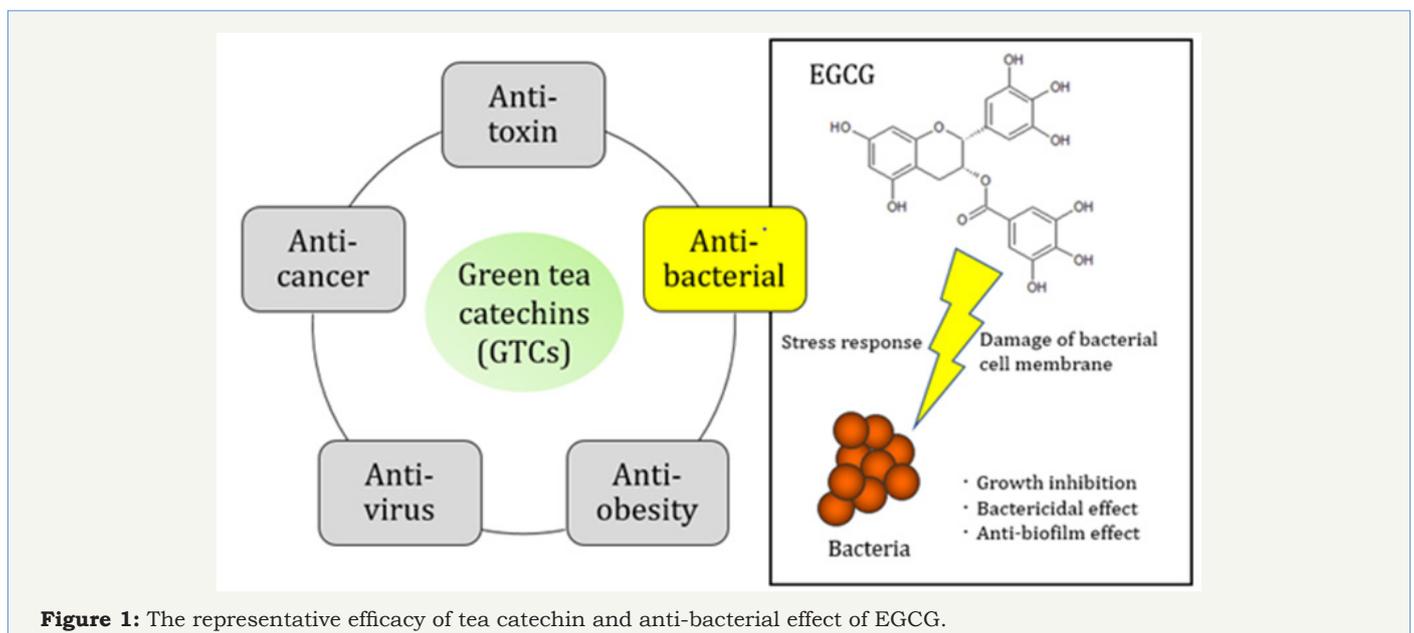
Abstract

The anti-bacterial effects of green tea catechins (GTCs) have been extensively studied. (-)-Epigallocatechin-3-O-gallate (EGCG), the most abundant in GTCs, has shown the highest anti-microbial effect among GTCs. This review focuses on anti-bacterial effects and veterinary use of GTCs and EGCG. EGCG shows inhibition of bacterial growth and bio-film formation, and bactericidal effect, resulting from stress response and damage of cell membrane. In veterinary use, feed addition of EGCG and TE brought health improvement to livestock.

Keywords: Green tea catechins; Anti-bacterial effects; Bio-film

Abbreviations: GTCs: Green Tea Catechins; EGCG: (-)-Epigallocatechin-3-O-Gallate; ECG: (-)-Epicatechin Gallate; EGC: (-)-Epigallocatechin; EC: (-)-Epicatechin; MIC: Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration; CFU: Colony Forming Unit; MRSA: Methicillin-Resistant *Staphylococcus Aureus*; EHEC: Enterohemorrhagic *E. Coli*; EC35G: (-)-Epicatechin-3,5-O-Digallate; TE: Tea Extract

Introduction



Tea from the leaves and buds of the plant *Camellia sinensis* is one of the most popular beverages. Green, oolong, and black tea are all obtained from the leaves through full non-fermentation, semi-fermentation, and fermentation, respectively [1,2]. Green tea has beneficial effects against various diseases such as cancer and others [3,4]. Figure 1 shows the representative efficacy of tea

catechin. Recently, antimicrobial resistance is an emerging problem worldwide. Antimicrobial usage in animal production is thought to be a contributing factor. A post-antibiotic era is instead a very real possibility for the 21st Century. Green tea extracts and the components including synthetic new poly-phenol could be useful for disease prevention and treatment in veterinary medicine.

Green tea, tea extracts and poly-phenol compounds such as (-)-Epigallocatechin-3-O-gallate (EGCG) show growth inhibition of both Gram-positive and Gram-negative bacteria *in-vitro* and *in-vivo*. Green tea catechins (GTCs) acts on microbes but also on enzymes and toxins indicating the compounds deactivate proteins. Infectious diseases are targeted in the study of GTCs [5]. EGCG has shown the highest biological activity among GTCs in most of the studies. This review will discuss briefly the anti-bacterial effects of GTCs and EGCG.

Green Tea Composition

Green tea contains several poly-phenolic compounds, including flavan and flavanol derivatives. Catechins are the most frequent and abundant poly-phenolic compounds [6]. The significant GTCs include EGCG and (-)-epicatechin gallate (ECG), which are produced by the esterification of (-)-epigallocatechin (EGC) and (-)-epicatechins (EC) with gallic acid. EGCG is the most abundant, accounting for 50% [6] to 65% [7] of total catechins.

Anti-Bacterial Effects

Anti-bacterial effects of tea and GTCs have been demonstrated against Gram-positive and Gram-negative bacteria including *Staphylococcus aureus*, *Vibrio cholerae*, *Escherichia coli*, *Shigella spp.*, *Salmonella spp.*, *Bacillus spp.*, *Klebsiella spp.* and *Pseudomonas aeruginosa* [8]. Tea extracts were also bactericidal to staphylococci and *Yersinia enterocolitica* [9]. EGCG inhibits the growth of *S. aureus* and *Escherichia coli* [10]. EGCG at the concentrations between 78 and 625 µg/mL inhibited multi-drug resistant *Acinetobacter baumannii* [11]. Various bacteria isolated from canine oral cavity were sensitive to the polyphenolic compounds mix and EGCG [12]. Minimum inhibitory concentration (MIC) ranges were 0.1-0.8mg/mL for poly-phenolic compounds and 0.0125-0.1mg/mL for EGCG. It is well-known that green tea is effective for caries control. *Streptococcus mutans*, which is a causative agent of caries, produces insoluble glucan and organic acids in the process of biofilm (plaque) formation. The growth of *S. mutans* was inhibited by EGCG, ECG, and EGC (the order of their inhibitory effect: EGCG>ECG>EGC), while EC did not show inhibitory function against *S. mutans*. The MIC and minimum bactericidal concentration (MBC) values of EGCG against *S. mutans* were 0.125 and 0.1mg/mL, respectively. Tea and tea ingredients showed not only anti-bacterial but also antifungal and antiviral properties.

Anti-Biofilm Effects

Sudano et al. [13] demonstrated that EGCG brought a decrease of slime production and inhibition of biofilm formation by ocular *S. aureus* and *S. epidermidis* isolates [13]. Moreover, the colony forming unit (CFU) of *S. mutans* obtained from biofilm formed in the presence of EGCG were less than that formed in the control culture. These indicate that EGCG had an inhibitory effect on biofilm formation [12]. It has been reported that in addition to binding to lipid layers and peptidoglycan, EGCG interferes with extracellular polymeric material so-called glycocalyx. GTCs significantly inhibit

mixed biofilm formation, and reduce the synthesis of auto inducer 2 which is signaling molecule used in quorum sensing [14]. EGCG can eliminate the bio-film matrix by inducing the σE cell envelope stress response and thereby reducing the expression of CsgD [15].

Interaction of Bacterial Cell Components

Initial experiments suggested that negatively charged EGCG exerts its anti-bactericidal activity by binding to the positively charged lipids of the bacterial cell membrane, causing damage to the lipid layer. Subsequently, the interaction of catechins including EGCG with lipid bilayers has been studied in more detail [16,17]. Results from Zhao et al. [10] indicated that EGCG binds directly or indirectly to the peptidoglycan of the bacterial cell wall and inhibits the penicillinase activity, protecting penicillin from inactivation [10].

In-vivo model

Steinmann et al. reviewed that the properties of EGCG in various trials against infectious diseases such as methicillin-resistant *Staphylococcus aureus* (MRSA), *Stenotrophomonas maltophilia*, *Helicobacter pylori*, *E. coli* and others [5]. We observed that gnotobiotic mice with GTCs had significantly lower Shiga toxin levels than the untreated control group after *Enterohemorrhagic E. coli* (EHEC) infection [18]. This report also includes the inhibition of bacterial growth *in vivo*. The untreated controls developed neurological and systemic symptoms, usually culminating in death, whereas none of the mice received green tea extracts exhibited any clinical symptoms or died. The combination of green tea extract and levofloxacin increased survival rates and reduced damage to target organs in orally EHEC infected gnotobiotic mice [19]. Thus, GTCs and EGCG has significant direct and indirect anti-pathogenic effects against food borne bacteria and other bacteria, including multi-drug-resistant strains.

Enhancement of Biological Activity by Chemical Modification

The functional and structural differences between these catechins are attributed to the number of hydroxyl groups on the B-ring and the presence or absence of a galloyl moiety. Some chemical modifications have been performed to enhance the biological activity of catechins. Galloyl modification is one of the method to enhance catechins [20]. Galloyl moiety is important to the activity of catechin, it is known that catechins without galloyl moiety have low anti-microbial activity [21]. It is expected that inducing galloyl moiety to original catechins could enhance anti-microbial activity. We demonstrated anti-microbial activity of (-)-epicatechin 3,5-O-digallate (EC35G) [22], galloyl modified catechin from EC. This catechin is made from EC by inducing two galloyl moieties to position 3,5. EC35G inhibited the growth of MRSA, but EC did not.

Use of Tea Extract (TE) for Food Products

The safety and quality of food products are important. The use of antimicrobial film is one of tools for contaminated pathogenic

bacteria. For example, incorporation of TE into chitosan-coated films enhanced their effectiveness against *Listeria monocytogenes* [23]. Packaging film using TEs could improve the safety of ham steak during room/refrigerated storage. Edible polymer coating with TE was had a significant effect in reducing the fat oxidation of chicken nuggets [24]. Wagh et al. [25] described that plant-derived extracts such as TE can be valuable to the modification of frankfurter formulations for improve oxidative stability [25]. Actually, TE showed strong antioxidant activity [26]. Thus, TE could

be useful for the safety from bacterial contamination and quality for inhibition of fat oxidation for meat industry and market.

Application of Catechins for Veterinary Science

Much attention was focused on biological activities of catechins in promotion of animals, because human have long applied catechins as dairy drinking. Several are already being tested for medial application. In veterinary science, TE has been used as supplement of feeding for livestock animals (Table 1) [27-33].

Table 1: The veterinary use of tea extract (TE) and EGCG.

| Animal | Compound | Application | Effective dose | Effects | Reference |
|---------|----------|---------------------------------|---------------------|--|-----------|
| Pig | TE | Feeding on dairy diet | 0.2% TE in the diet | Suppression of odor of feces | [27] |
| | TE | Feeding on water | 200~400µl/kg | Gut health status | [28] |
| Chicken | TE | Feeding on dairy diet and water | 10g/kg | Anti-influenza virus activity | [29] |
| | EGCG | Oral administration | 40~80mg/kg | Alleviation of fat deposition | [30] |
| | TE | Feeding on dairy diet | 125~500mg/kg | Antioxidant Immunostimulant | [31] |
| Goat | TE | Feeding on dairy diet | 5~20mg/kg | Good effects on growth and meat quality Immunostimulant | [32] |
| Cow | TE | Feeding on dairy die | 0.175g/kg | Energy-corrected milk Lower concentrations of triacylglycerols and ch-olesterol in the liver | [33] |

Hara et al. [27] demonstrated that feeding the diet supplemented with 0.2% of tea polyphenols to pigs for 2 weeks, the odor of the feces was reduced [27]. During tea polyphenols administration, the level of lactobacilli was increased whereas bacteroidaceae and clostridia were decreased. Moreover, fecal phenol, p-cresol, and skatole were reduced.

Supplementing broiler chicken diets with TE showed antimicrobial effects including anti-coccidial [34] and anti-influenza activity [29]. As long-term effects in chicken, tea polyphenols improved lipid metabolism and digestive enzymatic activities, and affected inflammatory status [35]. ECG supplementation significantly down-regulated the expression of fatty acid synthesis and β -oxidation/lipolysis genes [30]. They suggested that EGCG could alleviate fat deposition via the improved metabolism. Tea poly-phenols reduced glucocorticoid-induced growth inhibition and oxidative stress in chicken [36]. Tea poly-phenol was also effective to laying hens in the view point of anti-oxidant status, performance of reproduction and egg quality [37].

Mastitis in dairy cows is a disease of economic considerable importance [38]. *S. aureus* is the most common cases of mastitis in dairy cows. In Belgium, MRSA isolates obtained from bovine mastitis cases was first reported in the 1970 [39]. EGCG shows anti-bacterial effects to not only *S. aureus* but also MRSA. This suggests that EGCG is useful for treating mastitis caused by MRSA.

Conclusion

GTCs show anti-bacterial effect to Gram-positive and Gram-negative bacteria. This indicates that EGCG is useful for prevention and treatment of infectious diseases in domestic animals. Together

with the veterinary use described above, GTCs will be an excellent medicine that brings various efficacies to livestock.

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