

Anti-bacterial and Anti-toxin Activities of Catechins

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Background

Aggregatibacter actinomycetemcomitans (A.a.) is a Gram-negative bacterium that is associated with localized aggressive periodontitis. The bacterium can evade the host immune system in various ways including through the secretion of leukotoxin (LtxA). Catechins, natural compounds that make up 30% weight of green tea leaves, have demonstrated antimicrobial properties to both Gram positive and Gram negative bacteria as well as to some fungi and viruses. Recent studies show catechins' potential of being a natural defense against oral infections. However, the mechanism of this protection is unknown.

Here, the interactions between LtxA and catechin are examined. Six catechins were tested: catechin (C), epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECg), epigallocatechin gallate (EGCg), gallicocatechin gallate (GCg).

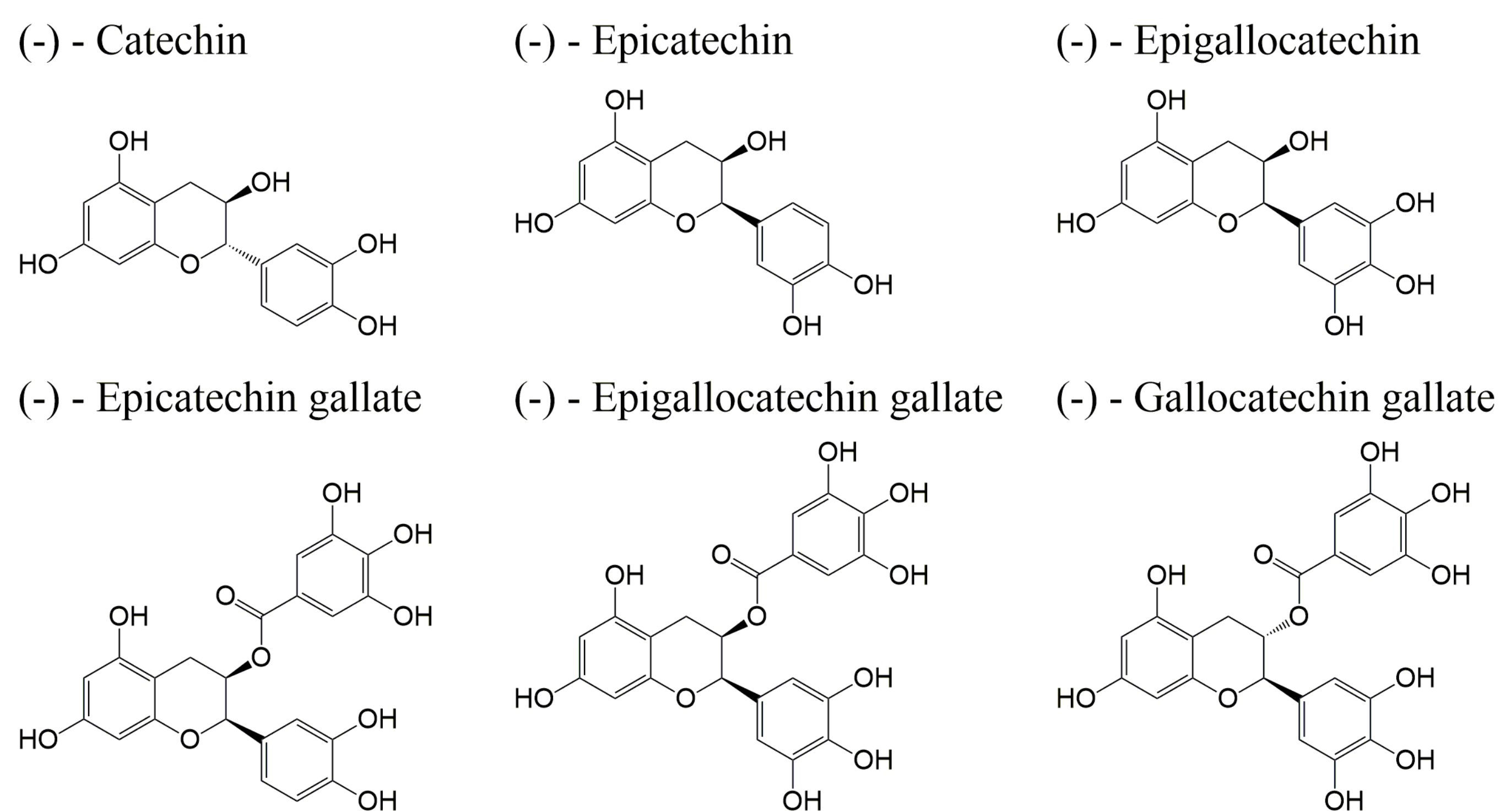


Figure 3. Molecular structures of the six catechins tested.

Methods

Confocal Microscopy

- Form giant unilamellar vesicles (GUVs) using POPC, 20% cholesterol, and NBD-PE (green)
- Dye LtxA with Alexa Fluor 646 (red)
- Incubate dyed LtxA with one of the catechins for 30 minutes
- Incubate pre-treated LtxA with GUVs

Cytotoxicity

- Pre-treat LtxA with one of the catechins for 30 minutes
- Incubate pre-treated LtxA with THP-1 cells for 3 hours
- Measure cell viability using Trypan blue assay

Circular Dichroism (CD) Spectroscopy

- Pre-treat LtxA with EGCg or C for 30 minutes
- Measure CD spectroscopy of LtxA

Conclusions

Catechins, especially galloylated catechins, induce a change in the secondary structure of LtxA. As a result, LtxA is unable to bind to its receptor, cholesterol, and is ineffective in killing its target cells.

Results

Five out of the six catechins were effective in preventing LtxA from binding to GUVs. Galloylated catechins were more effective in preventing binding than non-galloylated catechins. Incubation of LtxA with EGCg caused significant alteration of the secondary structure of LtxA. Results are consistent with the cytotoxicity experiment, where the galloylated catechin EGCg influenced LtxA more than non-galloylated catechin C.

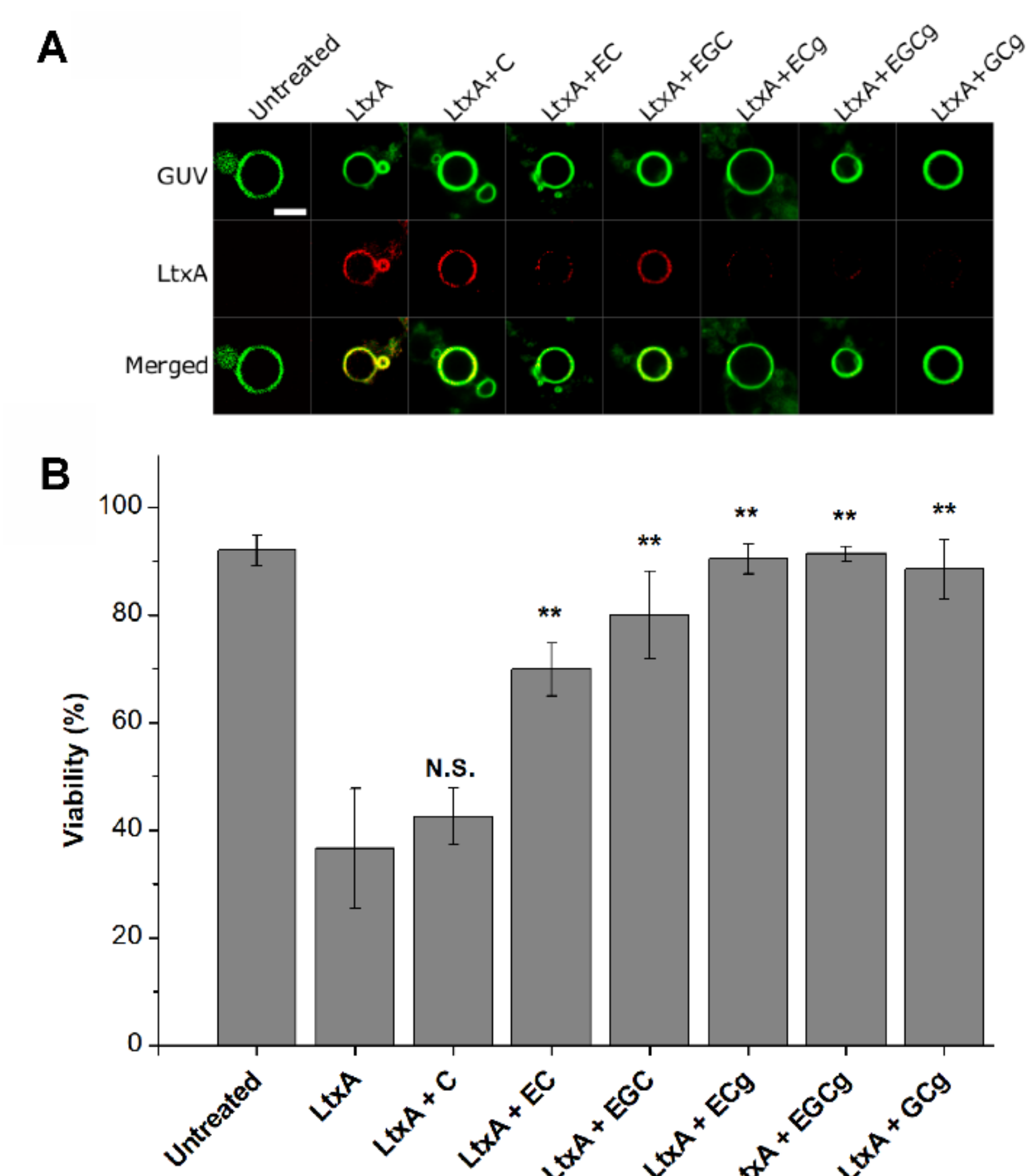


Figure 1. Interaction of LtxA with GUVs and THP-1 cells. A. Confocal microscopy images of LtxA (red) binding to GUVs (green) after incubation with catechins. B. LtxA was pre-treated with one of the catechins and then incubated with THP-1 cells. Cell viability was measured after 3 hours.

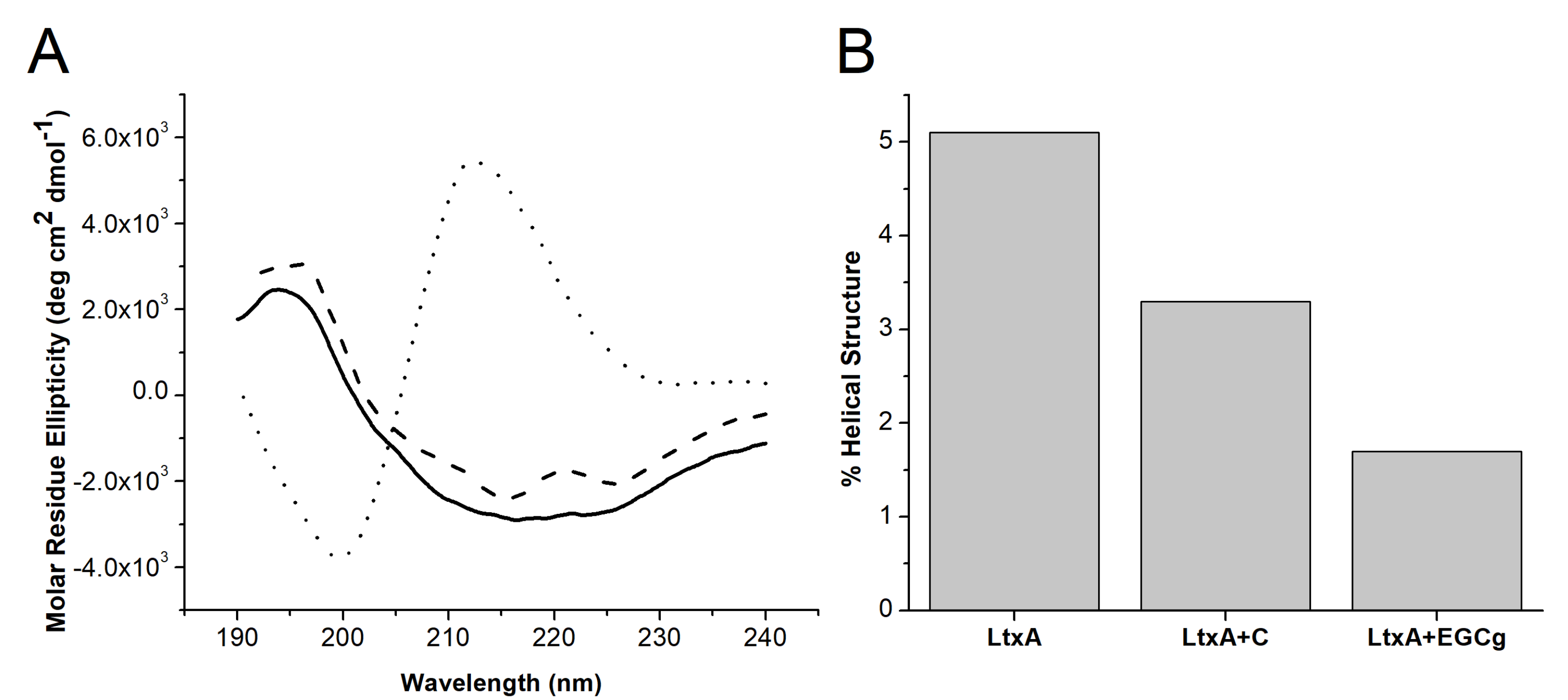


Figure 2. A. CD spectra of untreated LtxA (solid line), LtxA incubated with EGCg (dotted line), and LtxA incubated with C (dashed line). B. Helical structure of LtxA after treatment with catechin.

References

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