RAISE YOUR GLASS! AMERICAN CHESTNUT LEAVES CAN REDUCE TEA OXALATE CONTENT

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Intro/background:

The American chestnut tree (ACT, *Castanea dentata*), once considered the most important North American forest tree due to its expansive range and nut products, has nearly gone extinct due to "chestnut blight" caused by *Cryphonetria parasitica*. This fungus, an accidental import from Asia in the early 1900's, secretes oxalic acid at lethal levels and accelerates tissue decay on tree hosts, like the ACT, that do not have oxalate degrading enzymes. In the 1990s and through efforts of the American Chestnut Foundation, a genetically modified ACT was developed containing a wheat gene that codes for the enzyme oxalate oxidase (OxOx) and expresses it at high levels, making the genetically modified ACT resistant to blight. What does this have to do with the endourology society and kidney stone formers?

Kidney stones, primarily containing the mineral calcium oxalate, affect one in ten Americans and cost over 10 billion dollars annually to treat. Many stone formers excrete excessive amounts of oxalate in their urine due to the over-ingestion of oxalate from dietary sources. This diagnosis is typically made by testing 24 hours of urine using the OxOx enzyme extracted from 10-day old barley seedlings at yields of 1 unit/100 gm (Trinity Biotech® Oxalate Kit, commercial). In 2020, our laboratory obtained and then extracted 15 units/100 gm OxOx from ACT leaves – far more than the yield from barley seedlings. Although this finding is exciting, we propose further testing of ACT leaf OxOx activity and explore potential commercial venues for their leaves.

Tea, an aromatic beverage prepared from leaves of the *Camellia sinensis* shrub, is high in soluble oxalate, and its intake is generally discouraged in stone formers. In this study, we propose to investigate the oxalate content of some of the more popular teas (hot) and analyze whether the addition of ACT leaves (extract vs finely ground) can decrease soluble tea oxalate levels. Should ACT leaves have the ability to lower tea oxalate levels, therapy could be developed to reduce urinary oxalate levels in tea drinkers who are at high stone risk. Additionally, adding ACT leaves to tea could provide commercial incentive to help restore this lost ecosystem of North America.

Methods:

A total of 6 teas were brewed per the manufacturer's recommendations (Yorkshire, Lipton, Tazo, PG Tips, Twinnings, and Greenwise) using calcium/magnesium free

phosphate buffered saline (PBS). Distilled and spring water was also used with Lipton teas to assess the difference in oxalate reduction between the brewing solutions.

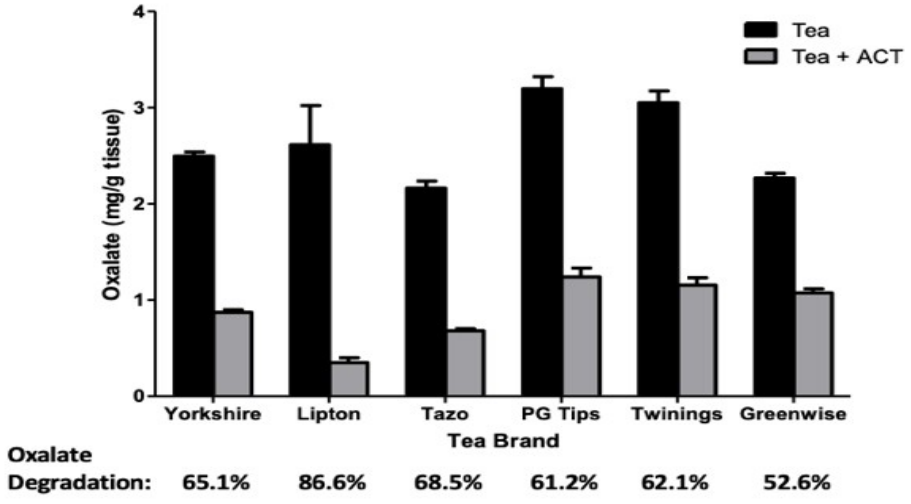
To assess oxalate reduction, we first measured the amount of oxalate content in 250mg of tea leaves without ACT leaves. Then, 250mg of tea with 250mg of ACT leaves were steeped in 25ml of boiling PBS and/or water for 5 min. Oxalate was measured using the Trinity Biotech Oxalate Assay.

To analyze the data, we calculated the mean milligrams of soluble Oxalate (mgOx) per sample of 0.25g of each tea's leaves ("tissue"), both with and without the additional 0.25mg ACT leaves. Doing so allowed us to assess the potency of each tea at a standardized level. However, for a more practical analysis of the data, we also calculated the mean mg Ox per serving size (varies according to the manufacturer), with and without the additional 0.25mg ACT leaves. The latter allows for the assessment of oxalate consumption when ingesting the tea as directed on each tea's box.

Results:

When viewing each tea, the consistent theme was that oxalate levels were considerably lower after steeping the tea leaves with ACT leaves. We found that before adding ACT leaves, there was a range of 2.16 to 3.19 mg Ox per g of tea leaves (Fig. 1), with Tazo being the lowest amount of soluble oxalate per gram of ACT leaves, and PG Tips being the highest. When ACT leaves were steeped with the tea, soluble oxalate was reduced by an average of 66.02%, with Greenwise being the lowest reduction at 52.6% and Lipton having the highest reduction at 86.6%.

Figure 1: Soluble Oxalate Per Gram of Tea



When viewing the data in terms of mg Ox per serving sizes of the tea leaves, we see a similar trend once the ACT leaves are added (Fig 2). We found that before adding

ACT leaves, there was a range of 5.1 to 9.3 mgOx per serving size, with PG Tips being the highest and Greenwise being the lowest amount of soluble oxalate per serving. Again, this view of the data allows us to analyze how much soluble oxalate will be ingested if the tea is consumed per the brand's direction.

Figure 2: Soluble Oxalate Per Serving of Tea

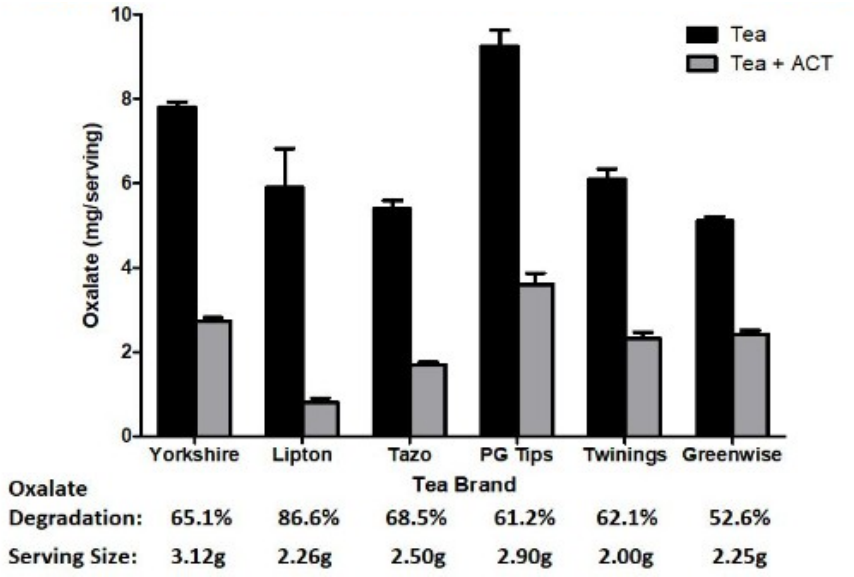
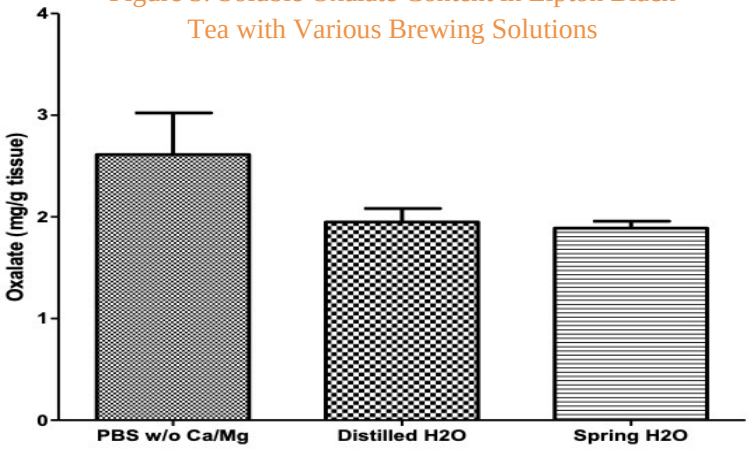


Figure 3 shows data from Lipton tea when steeped with PBS, distilled water, and spring water. We see a difference of 0.672 mg Ox per gram of tissue between the PBS and the distilled water, and a difference of 0.732 mg Ox per gram of tissue between the PBS and the spring water. These results indicate that there is not a significant difference in the oxalate levels between the different solutions that were used to brew the tea.

Figure 3: Soluble Oxalate Content in Lipton Black Tea with Various Brewing Solutions



Conclusion/future direction:

Dietary oxalate reduction is an essential element of the strategy to reduce the prevalence of renal stones in high-risk individuals. Teas are known to contain high concentrations of oxalate, and the goal of our study was to assess for a potential therapeutic role for American Chestnut Tree leaves in oxalate reduction of commercially available teas. Oxalate content in 6 commercial teas was reduced by an average of ~70% by the addition of ACT leaves, demonstrating a possible therapeutic role in helping to prevent renal stones. American Chestnut leaves alone, or in combination with other tea leaves, may mitigate the stone risk in stone formers who wish to enjoy the health benefits of freshly brewed tea.

Future work will focus on determining ACT OxOx enzyme kinetics and biochemical properties such as thermostability and pH optima. These data will be helpful to determine suitable conditions and tea/ACT leaf ratios for efficient oxalate degradation. Continuing this research will allow us to maximize the biochemical effect of the ACT leaves and provide the most benefit for patients who may use this as preventative treatment down the road.

References:

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