HYDROGEN PEROXIDE METABOLISM IN VIVO

Yee Pinn Tsin\(^1\) and Halliwell, Barry.\(^2\)

Department of Biochemistry, Faculty of Science, National University of Singapore
10 Kent Ridge Road, Singapore 117546.

ABSTRACT

A recent study discovered that consumption of green tea decreased urinary H\(_2\)O\(_2\) levels, while coffee increased the H\(_2\)O\(_2\) content. As both beverages did not contain the same content of H\(_2\)O\(_2\), valid comparisons could not be made. This study was carried out to observe the effects on urinary H\(_2\)O\(_2\) by using green tea and coffee containing the same amount of hydrogen peroxide, within the range of 42-44 µM. This was demonstrated using the ferrous ion oxidation-xylenol orange (FOX) and Amplex red assay. Theoretically two solutions with the same H\(_2\)O\(_2\) content might be expected to produce similar results, but tea and coffee still elevated and reduced levels of H\(_2\)O\(_2\) in urine respectively. Addition of milk into coffee also reduced urinary H\(_2\)O\(_2\). We conclude that tea, coffee and milk contain certain compounds that, upon excretion in urine, work synergistically or antagonistically to eliminate or scavenge urinary H\(_2\)O\(_2\). Thus these beverages illustrate a complex and intricate mixture of anti- and pro-oxidant abilities.

INTRODUCTION

There has been considerable interest in the beneficial effects of certain beverages such as green tea, red wine and black tea. These beverages are rich in polyphenolic compounds especially flavonoids. However, these beverages have also been shown to be mutagenic in bacterial test systems. Hence, more attention has to be given to the mutagenicity of some of these beverages. Upon addition of catalase, this mutagenicity was eliminated; hence it appears to be due to H\(_2\)O\(_2\) generation. We have investigated the H\(_2\)O\(_2\) generating capabilities in several beverages by directly measuring its production in samples of green tea, instant coffee and coffee with pasteurized milk. Upon standing for 2 hours, all beverages showed an increase in H\(_2\)O\(_2\) content at different rates. Coffee and milk displayed the lowest initial concentration of H\(_2\)O\(_2\).

A recent study by one of Prof Halliwell’s Honours student elucidated that green tea was capable of decreasing urinary H\(_2\)O\(_2\) levels. In contrast, instant coffee showed significant rises in urinary H\(_2\)O\(_2\) levels. Hence, this leads to the reduction in basal urinary H\(_2\)O\(_2\) levels. Coffee contains predominantly monophenolic compounds and less catechins like in tea. This beverage contains relatively higher concentration of mutagens like methylglyoxal and H\(_2\)O\(_2\) than in tea, it is unsurprising that coffee consumption would lead to a rise in H\(_2\)O\(_2\) levels. This was further proved in

\(^1\) Student
\(^2\) Professor
the study that shows rapid and reproducible elevated levels of urinary H\textsubscript{2}O\textsubscript{2} up to 2 hours after drinking the beverage.

**MATERIALS AND METHODS**

Nine healthy males and females between the ages of 20 to 40 years old were recruited. They were non-smoking and had a BMI within the normal range of 18.5 – 21. The subjects had no prior history of diabetes, hypertension, cancer, liver or heart disease. All subjects were restricted from consuming any beverage besides plain water throughout the day of sample collection. They were not required to fast or undergo strict dietary guidelines.

Initial efforts were made to determine the approximate weight of coffee that would have the same H\textsubscript{2}O\textsubscript{2} content as in the cup of green tea used in this experiment. The H\textsubscript{2}O\textsubscript{2} concentration in both coffee and tea were made to be approximately the same, within the range of 42-44 µM. A combination of different assays was used in this study such as the ferrous ion oxidation-xylenol orange (FOX), Amplex Red and creatinine assay. As drinking fluids alter the water balance in the urine and hence the H\textsubscript{2}O\textsubscript{2} content, all levels were corrected against creatinine.

In the presence of horseradish peroxidase, Amplex Red oxidises H\textsubscript{2}O\textsubscript{2} into a red fluorescent compound, resorufin that has an excitation wavelength at 563 nm and emission wavelength at 587 nm.

**RESULTS AND DISCUSSION**

![Graph](image-url)

**Fig 1:** Effects of green tea and coffee on urinary H\textsubscript{2}O\textsubscript{2}. Results are expressed as fold changes relative to the initial H\textsubscript{2}O\textsubscript{2} concentration due to large differences in initial concentrations. Data are presented as mean ± SD (n=3).
Fig 2: Effects of coffee and milk on urinary H$_2$O$_2$ levels. The results are expressed as fold changes with H$_2$O$_2$ reference to the initial time point. Studies were repeated at least 3 times on different days. Data are represented as mean ± SD (n=3).

It was discovered that tea still decreased and coffee increased urinary H$_2$O$_2$ levels. This shows that tea contains certain protective components that not only prevent the H$_2$O$_2$ from showing up in urine but also even to the extent of decreasing the basal levels of H$_2$O$_2$. Coffee still produced a prominent peak within 2 hours of consumption. H$_2$O$_2$ reduction caused by the addition of milk into coffee was greater than what might have been expected. This decline was comparable to that observed in tea. Milk should only have reduced H$_2$O$_2$ in coffee and not reduced the urinary basal levels.

We wanted to observe whether the same results could be obtained when the two beverages were standardized to contain the same H$_2$O$_2$ content. We cannot omit the possibility that a very small percentage of H$_2$O$_2$ in the urine could have originated directly from the beverages in an unchanged form.

Nevertheless, most of the H$_2$O$_2$ would have been degraded in the gastro-intestinal (GI) tract as the human body might upregulate the antioxidant defense systems in response to H$_2$O$_2$. Besides that some salivary peroxidase in the buccal cavity could easily eliminate any H$_2$O$_2$ consumed. We are still unsure of the exact origin of urinary H$_2$O$_2$. Nonetheless, previous studies have shown a positive correlation between respiratory diseases like asthma and ARDS with high levels of urinary H$_2$O$_2$. As such, whether this ROS could be used as an invaluable biomarker of oxidative stress remains to be validated by further studies. This is because the levels of H$_2$O$_2$ in the urine is so dependent on environmental factors, genotype, individual’s metabolism and a multitude of other factors that could influence H$_2$O$_2$ concentration.

However, a more plausible method was put forward whereby plasma levels of H$_2$O$_2$ could be measured after consumption of tea or coffee to determine whether it has been absorbed into the GI tract. In this study it was shown that coffee, which contained the same amount of H$_2$O$_2$ as tea, still
elevated $\text{H}_2\text{O}_2$ levels. Thus, we proceeded to add pasteurized milk into coffee. Milk is capable of scavenging $\text{H}_2\text{O}_2$ and with this knowledge we wanted to observe the levels of urinary $\text{H}_2\text{O}_2$ upon drinking coffee with milk. The same nine subjects were recruited and had their urine samples taken at every hour interval. They were subjected to the same set of protocols as earlier on, but this time the beverage was coffee with milk.

It was interesting to note that the addition of milk into coffee showed a decrease in urinary $\text{H}_2\text{O}_2$. This was astounding as although milk could scavenge some of the Reactive Oxygen Species (ROS), coffee was still present in the beverage and hence, could still exert its mutagenic effects. We were expecting to see a rise in $\text{H}_2\text{O}_2$ levels but with a less significant peak as compared to coffee alone. This shows that milk contains certain compounds that could lead to the effects observed. This could not be attributed to active catalase as the heating treatment would have denatured the enzyme. The factor that sets milk apart from tea and coffee is the fact that milk contains hardly any $\text{H}_2\text{O}_2$, hence this could possibly contribute to the decrease in urinary $\text{H}_2\text{O}_2$. Further investigations need to be carried out in order to validate this.

Many in vitro studies have established tea’s role as an effective anti-carcinogenic and anti-tumourigenesis agent. However, there remains little evidence on tea’s effect in humans. More research needs to be carried out on tea and coffee’s dual role as a mutagen (pro-oxidant) and antioxidant. The interaction between milk and coffee should also be elucidated as to how the combination of these two beverages could display the $\text{H}_2\text{O}_2$ lowering effects similar to that of the single beverage, green tea.

ACKNOWLEDGEMENTS

The author is grateful to Prof. Barry Halliwell for his critical reading of the original version of this manuscript, and would also like to thank Dr Edward Jenner for his valuable suggestions. The author also wishes to acknowledge all the seniors and lab technicians in the Antioxidant Lab for their kind advice and encouragement.

REFERENCES


