

# A Study of the Genotoxicity of Caffeine Using the Comet Assay

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Globally, caffeine ranks as the most widely consumed drug, 97% of which is consumed in beverages (2012 Laszlo). In the past three decades a rise in soda consumption has coincided with a three-fold increase in esophageal cancer (2006 Boyles). Further investigation, however, has revealed an inverse relationship (2006 Mayne, 2010 Johnson). Some researchers cite caffeine as responsible for the supposed protection while others state that caffeine inhibits DNA synthesis and repair (2008 Nafisi, 1997 Tempel). Testing the two-sided alternative that caffeine affects DNA damage, this project explored the genotoxicity of caffeine. An alkaline comet assay, or single cell gel electrophoresis, assessed DNA damage in cat and cow liver cells harvested immediately after euthanization. Cells were split and assigned to one of the following treatments: a hydrogen peroxide control, a negative control (1X PBS), caffeine, benzene, or benzene + caffeine; n= 96, 117, 53, 101, and 93 respectively. Analysis of the controls verified the assay worked. At the  $\alpha = 0.05$  level, a Dunnett's Procedure comparing all treatments to the negative control suggests that caffeine did affect DNA damage, as analyzed by tail moment. Due to the significant P value of the caffeine group (0.0039), there is sufficient evidence to reject the null hypothesis that there is no difference in DNA damage between the cells treated with caffeine vs. a negative control and to conclude that caffeine increases DNA damage. Relative to the control, cells treated with benzene vs. benzene + caffeine yielded similar results. The results do not support the hypothesis that caffeine may protect against cancer. Further research is needed to assess the relationship between caffeine consumption and cancer incidence.