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Genotoxic effect of blood enhancement chemicals detected by the comet assay: A versatile tool that continues to expand its perspectives

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Phenolphthalein and Tetramethylbenzidene (TMB) are the most sensitive blood enhancement chemicals used as blood presumptive test in forensic laboratories. In these test, reduced form of colorless chemicals become oxidized when it reacts with hydrogen peroxide  $(H_2O_2)$  and visual color change has been observed. Here in these test,  $H_2O_2$  catalyzed the reaction. Additionally, it is also well known that  $H_2O_2$  induces DNA degradation in cellular system. In consequences, presence of  $H_2O_2$  in the presumptive test, indeed affect the further DNA analysis and result interpretation. Therefore, fate of DNA in the blood sample exposed to blood enhancement chemicals are urgently needed. To assess the fate of DNA, in the present study a well-known molecular biology technique that is single–cell gel electrophoresis assay which is also known as comet assay has been introduced. This is a rapid and sensitive assay used for the detection of DNA degradation. Our results exhibited that both blood enhancement chemicals (Phenolphthalein and TMB) alone and combined with  $H_2O_2$  induce DNA degradation in blood sample exposed to it as compared to unexposed control sample. Therefore, blood sample on which presumptive test (Phenolphthalein and TMB) has been performed, cannot be used for further DNA analysis. Our study not only reveals the genotoxic effect of blood enhancement chemicals on blood samples but also attempts to explore the application of well-known molecular biology technique that is comet assay in the field of forensic science. This assay will open a new opportunity in the field of forensic research and development.

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