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Abstract
Caffeine as in (coffee, cola, and tea) is the most widely consumed beverages worldwide. The current study aims to evaluate the effects of caffeine in different concentrations on human cultured peripheral lymphocytes, in healthy individuals, using comet assay. The extent of DNA damage reflects a balance between oxidative stress (the presence of hydrogen peroxide H2O2 as a reactive oxygen species ROS), and DNA repair ability (the presence of anti-oxidant may be caffeine substances at known concentrations). This is an important method to prevent and avoid many cancerous diseases in an era of various pollutants.


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Original Article
EVALUATING THE LEVELS OF OXIDATIVE DNA DAMAGE IN HUMAN LYMPHOCYTES IN RESPONSE TO CAFFEINE USING COMET ASSAY (SINGLE CELL GEL ELECTROPHORESIS)

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Received: 09 Feb 2016 Revised and Accepted: 20 Apr 2016
OBJECTIVES

Caffeine as in coffee, cola, and tea is the most widely consumed beverages worldwide. The current study aims to evaluate the effects of caffeine in different concentrations on human cultured peripheral lymphocytes, in healthy individuals, using comet assay. The extent of DNA damage reflects a balance between oxidative stress (the presence of hydrogen peroxide H2O2 as a reactive oxygen species ROS), and DNA repair ability (the presence of anti-oxidant may be caffeine substances at known concentrations). This is an important method to prevent and avoid many cancerous diseases in an era of various pollutants.

METHODS: Ten milliliters of venous blood samples were collected from 40 healthy young individuals, and lymphocyte cultures were set up after lymphocyte isolation with ficoll centrifugation. The mixture of lymphocytes culture media was incubated in the sterile incubator for 5 min after adding serial concentrations of caffeine (100, 500, 5000, 10000) μg/ml, as(group 1, 2, 3, 4 respectively) to 5% H2O2. The levels of oxidative DNA damage were expressed as comet tail length.

RESULTS: At concentration 100 μg/ml, there was a significant elevation in the mean comet tail length level in cultured lymphocytes treated with hydrogen peroxide (106.96 μm) compared with the treated with All (mixture of caffeine, and H2O2), 6.670 μm.

CONCLUSION: We’ve concluded that a caffeine concentration of 100 μg/ml possesses the strongest anti-oxidant properties and causes much less DNA damage in lymphocytic culture when exposed to hydrogen peroxide.

KEYWORDS: Oxidative DNA damage, Comet assay, Lymphocyte, Hydrogen peroxide

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The level of DNA damage in human cultured lymphocytes will be evaluate, in presence of Beta-Carotene as (antioxidant materials) in different concentrations, and to investigate the effect of Beta-Carotene on the stability of DNA and its effects on apoptosis may occur in these cells.

World Journal of Pharmacy and Pharmaceutical Sciences

Volume 5, Issue 3, 257-266.

www.wjpps.net

Evaluation of the degree of oxidative DNA damage & apoptosis in human lymphocytes cultured in the presence of Beta– Carotene using Comet assay, and Fas L(CD95).

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Abstract:

Background: The level of DNA damage in human cultured lymphocytes will be evaluate, in presence of Beta-Carotene as (antioxidant materials) in different concentrations, and to investigate the effect of Beta-Carotene on the stability of DNA and its effects on apoptosis may occur in these cells.

Aim of the study: To assess the levels of DNA damage and to measure the proportion of the DNA cellular repair in human lymphocytes cultured in vitro conditions, the impact of the presence of Beta-Carotene using Comet assay (single cell gel electrophoresis), and to evaluate the apoptosis in these cells if it may occur.

Subjects and Methods: The study inclusion 50 individuals aged between 20-50 years, during the period from 12 October 2014 to 19 November 2015 and from individuals with no family history of any disease, non smokers, and non conception of any type of vitamins, since the 1-2 weeks of sampling. Ten milliliters of total blood sample taken to hepernized container 10 sample (5 male, 5 female) to study the effect of different concentrations of Beta-Carotene (100 and 10000) µg / ml on cultured lymphocyte by trazoleom assay; then take another samples (40) to assess the level of DNA damage in cultured lymphocytes by single cell gel electrophoresis in presence of the two different concentrations of Beta-Carotene (100 and 10000) µg / ml, and these same samples used for estimation of Fas L(CD95).

Results: There were a damage occurring in DNA of the cultured lymphocytes by the effect of the presence of hydrogen peroxide, there were repair occurring by the presence of Beta-Carotene in the concentrations (100 and 10000 micrograms / ml) and there were a protective effect to the DNA of cultured lymphocytes, and there were also a significant change in the average tail moments (in Comet assay) and as an indicator of a positive effect for Beta-Carotene to protect DNA of the cultured lymphocyte cells. There were no detectable levels of FasL(DC95) in cultured peripheral
blood lymphocytes of healthy individuals, and there were no expression of FasL(CD95) in these cells, and there were no stimulation for the healthy peripheral blood lymphocytes by Beta Carotene in different concentrations (100,10000) µg/ml to expression of FasL(CD95).

**Conclusions:** The Comet assay is important assay to assess the damage and repair of DNA in human cultured lymphocytes in presence of Beta-Carotene, which showed that the most effective concentration of Beta-Carotene as antioxidant was in the concentration of 10000 µg / ml, as well as for that when were a combination of Beta-Carotene and hydrogen peroxide the most effective concentration was also in 10000 µg / ml, there were no stimulation for the healthy peripheral blood lymphocytes by Beta Carotene in different concentrations (100,10000) µg/ml to expression of FasL(CD95).

**Keywords:** Beta Carotene, lymphocytes, comet assay.

World Journal of Pharmacy and Pharmaceutical Sciences

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