

# A Comparative Study of Susceptibility of HepG2 and HaCat cells to DNA Damage

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**Abstract—** Background: Hydrogen peroxide is a natural source of oxidative DNA damage in cells generate reactive oxygen species (ROS), which are responsible for DNA damage and apoptosis. Aim: We investigated the susceptibility of HepG2 and HaCat cells to DNA damage capacity induced by hydrogen peroxide, as well as to compare the differential sensitivities of both types of cells to DNA damage. Methods: HepG2 and HaCat cells were challenged with 25  $\mu$ M hydrogen peroxide for 5, 30, 40, 60 min and 24 hr using Comet assay. Results: DNA damage was significantly increased in both HepG2 and HaCat cells at all times of incubation ( $p < 0.001$ ), but not at 24hr. Furthermore, there was a clear decrease in the amount of DNA damage was observed at 24 hr time of incubation, which is accompanied with decrease in DNA migration length in both HepG2 and HaCat cells. Conclusion: Our results confirm that the profile of DNA damage induced by hydrogen peroxide was similar in both HepG2 and HaCat cells. However, the reduction in the amount of DNA damage could be due to of possibility of cellular DNA repair.

**Index Terms—** DNA damage, hydrogen peroxide ( $H_2O_2$ ), HepG2 cells, HaCat cells, Comet assay.

## I. INTRODUCTION

A Reactive oxygen species (ROS), such as superoxide anion ( $O_2^-$ ), hydrogen peroxide ( $H_2O_2$ ), and the hydroxyl radical ( $OH\cdot$ ), are generated in vivo from the incomplete reaction of oxygen during aerobic metabolism, or from exposure to environmental agents such as radiation, redox cycling agents [1, 2]. The main reactive species responsible for oxidative DNA damage in cells appears to be hydroxyl radicals generated by hydrogen peroxide via the Fenton reaction in the presence of reactive transition metal ions, such as  $Fe^{+2}$  [3].

Cells must maintain a proper balance between the levels of free radicals, such as reactive oxygen species, and antioxidants. Generation of reactive oxygen species in

response to oxidative stress inducing damage to DNA, lipids and proteins. If the production of oxidizing agents such as free radicals and reactive oxygen species exceeds the capacity of cellular antioxidants in a biological system oxidative stress introduced [4]. Mammalian cells contain antioxidant mechanisms to scavenge or neutralize potentially damaging free radicals. These antioxidant mechanisms are enzymes such as superoxide dismutase, catalase and glutathione peroxidase are considered to be very important [5, 6]. Catalase or glutathione peroxidase converts hydrogen peroxide to water and oxygen [7]. Also, repair processes are remove free radical induced lesions in DNA. Accumulation of unrepaired DNA may be introduced mutation which has been implicated as the cause of some kinds of cancer and aging processes (8).

The single cell gel electrophoresis, also called the comet assay, has rapidly become one of the most popular and widely used methods since its introduction by Ostling and Johanson [9] and its independent modification by Singh et al. [10]. The comet assay is a simple, rapid, and sensitive technique for detecting DNA damage at the level of individual eukaryotic cells. The types of DNA damage that can be observed with this method are DNA double-strand breaks (DSB) and single-strand breaks (SSB) [11]. The Comet assay is used for a wide range of applications including DNA damage and repair studies [12], genotoxic studies, cell biological studies, human biomonitoring studies and nutritional research [13].

The purpose of this study was to investigate and compare the susceptibility of both HepG2 and HaCat cells to DNA damage capacity induced by 25  $\mu$ M hydrogen peroxide at different time of exposure.

## II. METHODOLOGY

This study was performed at Newcastle University, Institute for Research on Environment & Sustainability and Medical Toxicology Centre.

### A. Chemicals

Comet Lysis Buffer [2.5M Sodium Chloride, 100mM EDTA (pH 8.0), 1% Sarkosyl, 10 mM Tris-HCL (pH 8.0), 10% DMSO, 10% Triton X-100]. Comet Alkali Solution [0.3M Sodium Hydroxide, 1mM EDTA]. Comet Neutralising Buffer [0.5 M Tris-HCL (pH 7.5) Cryopreservation Medium [50% Foetal Calf Serum, 10% DMSO, 40% Dulbecco's Modified Eagle's Medium]. Resuscitation Medium [50% Foetal Calf Serum, 10% Dextrose, 40% Dulbecco's Modified Eagle's Medium]. Culture Medium for HepG2 and HaCat

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cells [Dulbecco's Modified Eagle's Medium (DMEM), 10% Foetal Calf Serum, 50 Units/ml Penicillin, 50Units/ml Streptomycin, 2 mM L-Glutamine]. All the previous materials were obtained from Sigma chemicals Co, Dorset UK.

**B. Cell culture**

HepG2 and HaCat cells were obtained from the European Cell Culture Collection (ECCC, UK). HepG2 cells are human Caucasian hepatocyte carcinoma cells taken from a primary hepatoblastoma (liver biopsy) from an 11-year-old male from Argentina in 1979. HaCat cells are an epithelial keratinocyte cell line that was isolated from the periphery of a melanoma on the upper half of the back of a 62-year- old male in 1988.

**C. Hydrogen peroxide treatment**

Cells plated into multi-well plate in a 1mL cell suspension concentration of (5x106 cells/ml) in Dulbecc's Modified Eagle's (DMEM) supplemented. The cells were allowed to attach for 24hr. Then the medium was removed and followed by addition 25 µM of hydrogen peroxide prepared from a stock solution 8 mM in water. Cells were incubated for 5 min, 30 min, 40 min, 1 hr, and 24 hr. For each time of incubation duplicate wells were used for HepG2 and HaCat cells. Wells were then used for Comet assay analysis.

**D. Comet assay**

The principle of the assay is based upon the ability of denatured, cleaved DNA fragments to migrate out of the cell under the influence of an electric field, whereas undamaged DNA migrates slower and remains within the confines of the nucleoid when a current is applied. Evaluation of the DNA comet tail shape and migration pattern allows for assessment of DNA damage.

The DNA damage was measured using Comet assay and expressed as olive tail moment (OTM); is the product of the tail length and the fraction of total DNA in the tail. The mean±SEM for the 50 cells (25 per slide) was recorded as our DNA damage data in this project. SYBR green dye was added to each slide to stain the single strand DNA, and then covered with a coverslip. The slide was visualised using either a BioRad MRC 600 Confocal microscope or a Leica TCS SP2 UV confocal microscope. The comet images were analysed using the kinetic komet version 5.5 software.

**E. Cell viability**

To determine cell viability an aliquot of 100 µL cell suspension was mixed 1:1 with trypan blue stain (0.4% w/v, Biowhittaker). Cells pipetted into a Neubauer haemocytometer counting chamber and visualised. In this study cell viability using trypan blue, was found to be over 95% at each time point of the study [14].

**F. Statistical analysis**

All data assumes Gaussian distribution, are expressed as mean±SEM. One-Way ANOVA was performed when more

than two groups were compared with a single control and the differences between individual groups were assessed by a Dunnett post hock, using Prism software (version 4).

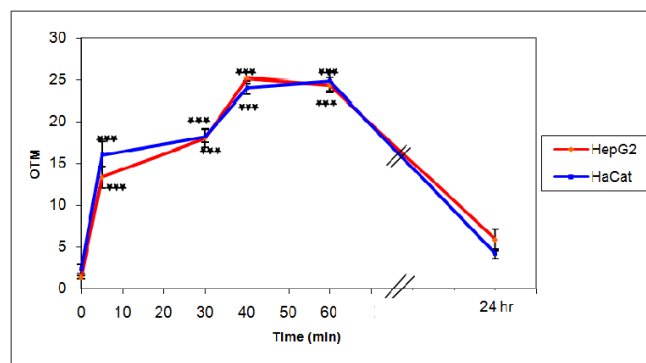


Fig 1:Effect of hydrogen peroxide and length of incubation on DNA damage forHepG2 and HaCat cells treated with 25 µM hydrogen peroxide for 5, 30, 40, 60 min and 24 hr. The values are expressed as OTM, mean±SEM for 50 cells per parameter (25 cells per slide).

\*\*\*P<0.001 (One-Way ANOVA) compared to control.

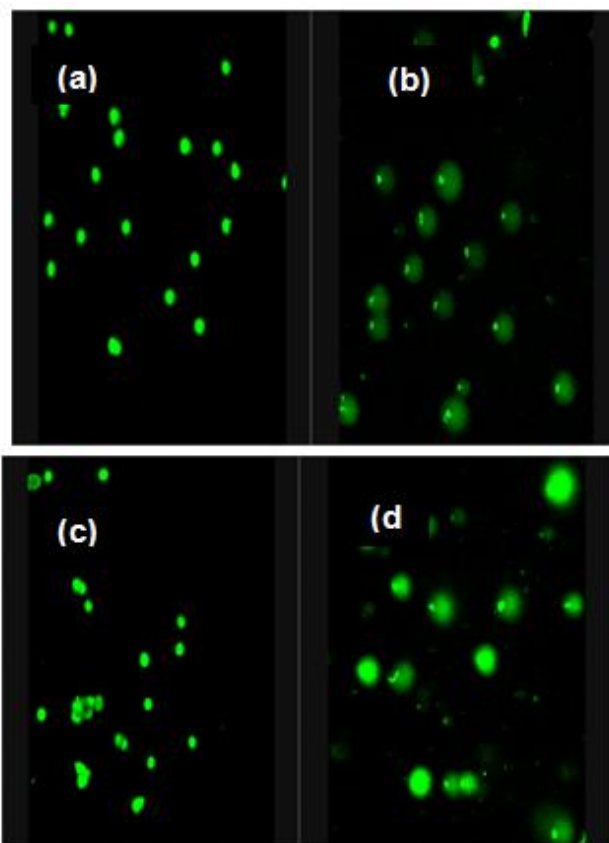


Fig 2: Effect of 25 µM hydrogen peroxide exposure on DNA damage for HepG2 and HaCat cells (a) control HepG2 cells (b) highest DNA damage induced at 5min time of exposure for HepG2 cells(c) control HaCat cells (d) highest DNA damage induced at 5min time of exposure for HaCat cells.

**Table 1:** DNA damage in HepG2 and HaCat cells treated with hydrogen peroxide 25  $\mu$ M for 5 min, 30 min, 40 min, 1 hr and 24 hr using comet assay. Cells expressed as olive tail moment (OTM) in HepG2 and HaCat cells all expressed as mean $\pm$ SEM, 50 cells were recorded per incubation (25 per slide, two pooled wells).

\*\*\*P<0.001 (One-Way ANOVA) compared to control.

Time	25 $\mu$ M hydrogen peroxide	
	HepG2 cells	HaCat cells
Control	1.4 $\pm$ 0.2	2.4 $\pm$ 0.5 ***
5 min	13.4 $\pm$ 1.3***	16.1 $\pm$ 1.5 ***
30 min	18.1 $\pm$ 1.1***	18.3 $\pm$ 0.8 ***
40 min	25.3 $\pm$ 0.7***	24.1 $\pm$ 0.8 ***
1 hr	24.4 $\pm$ 0.9***	24.9 $\pm$ 1.2 ***
24 hr	5.9 $\pm$ 1.3	4.2 $\pm$ 0.6

## II. RESULTS

In this study, the sensitivity of oxidative DNA damage in both HepG2 and HaCat cells was examined using hydrogen peroxide, significantly increased in DNA damage (P<0.001) was demonstrated in both HepG2 and HaCat cells (figure 1 and table 1) at 25  $\mu$ M hydrogen peroxide compared to untreated control cells at 5 min, 30 min, 40 min and 1 hr times of incubation.

But there were no significant differences observed between the levels of DNA damage (OTM) at 24 hr time of incubation compared to control cells in both HepG2 and HaCat cells.

Although in HepG2 cells and HaCat cells comparing the DNA damage profile using olive tail moment (OTM) was similar in both types of cells (figure 2). This indicates that the pattern of DNA damage was similar in both HepG2 and HaCat cells.

## III. CONFLICT OF INTEREST AND FUNDING

This work was funded by Ministry of Higher Education and Scientific Research, State of Libya.

## IV. DISCUSSION

Hydrogen peroxide is believed to be one of the most potent causes of DNA damage, chromosomal alterations, gene mutations and tumor promotion by generating of highly reactive and potentially dangerous hydroxyl radicals (OH $\cdot$ )

close to the DNA molecule by means of the Fenton reaction via transition metal ions such as ferrous ions: H<sub>2</sub>O<sub>2</sub> + Fe<sup>2+</sup>  $\rightarrow$  OH $\cdot$  + OH<sup>-</sup> + Fe<sup>3+</sup> [15, 16]. Cellular antioxidants appear to be crucial for the prevention of DNA damage induced by ROS which may lead to the respective diseases [17].

Rosignoli et al. [18] investigated that in human colonocytes 10  $\mu$ M hydrogen peroxide induced statistically significant dose-dependent effect of DNA damage using comet assay for 15 min. Cells being highly damaged with an increase in the concentration of hydrogen peroxide to 80  $\mu$ M. However, DNA damage was decreased with an increase in the exposure time of incubation, which is similar to our present study. In this recent study Piperakis et al. [19], compared the DNA damage in the lymphocytes of older and younger populations at 50, 100, 150  $\mu$ M hydrogen peroxide using comet assay produced a significant increase in DNA damage in a dose response relationship manner in all populations (p<0.05). At 2 hr time of incubation the lymphocytes DNA damage started to decrease which is lead to a DNA repair capacity.

Gasiorowski and Brokos [20] used human lymphocytes to assess DNA damage following treatment with 20  $\mu$ M hydrogen peroxide. Treatment of cells with hydrogen peroxide for five minutes induced DNA damage, while subsequent incubation of the cells for 2 hr lead to a decrease in DNA damage. Our results agree with the above-mentioned findings, as we observed increase in DNA damage in both types of cells (figure 2b and 2d) at 25  $\mu$ M hydrogen peroxide compared to control with decrease in DNA damage started at 1 hr time of incubation (table 1).

Similar results were reported by Natacha et al. [21] using rat thyroid cell line which was induced high levels of DNA damage at nonlethal hydrogen peroxide concentrations. Moreover, double strand breaks were induced in human thyroid in primary culture. Our findings demonstrate that the susceptibility and sensitivity of HepG2 and HaCat cells to the DNA damage induced by hydrogen peroxide were similar.

Comparing the DNA damage in HepG2 and HaCat cells at 25  $\mu$ M hydrogen peroxide, there was significant damage at all treated times and the highest DNA damage was observed between 5 and 60 min in both types of cell. After 24 hr DNA damage was decreased to similar levels to control; it could be due to the DNA repair in both of cells. The profile of DNA damage at 25 $\mu$ M hydrogen peroxide was similar in HepG2 and HaCat cells.

Henzler and Steudle [22] investigated that mechanism of DNA damage induced by hydrogen peroxide involved the reaction between Fe<sup>2+</sup> and hydrogen peroxide which is called the Fenton reaction. The hydroxyl free radicals were the primary oxidizing species and implicated in the mechanism of oxidative DNA damage. Hydrogen peroxide resembles the chemical properties of water, and thus uses water channels (aquaporins) to cross the cell membrane rapidly [23].

## V. CONCLUSION

we have demonstrated that HepG2 and HaCat cells showed higher sensitivity to hydrogen peroxide at 25  $\mu$ M. Moreover,

the profile of DNA damage induced was similar in both HepG2 and HaCat cells. Furthermore, Comet analysis parameter OTM has the equal prognostic capacity to measure the DNA damage in both HepG2 and HaCat cells. Comet assay technically is a simple and fast method that detects genotoxicity in virtually any mammalian cell type.

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