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# β-ESTRADIOL INDUCES CYTOTOXIC EFFECTS TO HUMAN T-LYMPHOMA (JURKAT) CELLS THROUGH OXIDATIVE STRESS

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## Abstract

β-estradiol is the most potent estrogen of a group of endogenous estrogen steroids which includes estrone and estriol. This steroid hormone is the most potent natural estrogen, produced mainly by the ovary, placenta, and in smaller amounts by the adrenal cortex, and the male testes. Although β-estradiol protects the renal and cardiovascular systems, the mechanisms involved remain unclear. In this research, we performed the MTT [3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay to evaluate the effect of β-estradiol on human T-lymphoma (Jurkat) cells upon 24 and 48 hours, respectively. Lipid peroxidation assay was also performed to estimate the levels of malondialdehyde (MDA) production in β-estradiol-treated cells. The results of MTT assay demonstrated that low, physiological levels of β-estradiol induce cellular proliferation in Jurkat T-cells. At higher dose of exposure, β-estradiol decreases the viability of Jurkat T-cells compared to the control cells. Data generated from lipid peroxidation assay resulted in a significant increase ($p < 0.05$) in MDA production in β-estradiol treated sample. Upon 48 h of exposure, MDA concentrations in the sample [μM] (mean ±SE, n = 3) compared to untreated control were 4.9 ± 1.7, 8.1 ± 1,6 11.5 ± 2.2, 21.1 ± 2.3, 19.5 ± 1.4, and 21.5 ± 2.6 in 0, 1, 2, 4, 8, and 16 μM β-estradiol, respectively. In summary, findings from this study demonstrated that high dose of β-estradiol is cytotoxic to Jurkat T-cells. This cytotoxicity is found to be associated with oxidative stress.

### Keywords

β-estradiol; MTT assay; Jurkat T-cells; Lipid peroxidation assay

## 1. INTRODUCTION

Estradiol is a form of estrogen, a female sex hormone produced by the ovaries. Estrogen is necessary for many processes in the body. In vitro studies indicated that the addition of estradiol to cultures of human lymphocytes decreases in CD4+/CD8+ T-cell-subset ratios (Athreya et al., 1993), and enhances immunoglobulin secretion (Kanda and Tamaki, 1999). Studies indicated that estrogens can affect cells of the immune system and may play a role in modulating lymphocyte development and function (Smithson et al., 1998, Grimaldi et al., 2002). Published reports suggest that estrogens may have important actions at other steps in the atherogenic process (Nathan and Chaudhuri, 1997). However, the basis mechanisms by which β-estradiol-induced cytotoxic effects on cancer cells are not yet completely

understood. In the present study, we use the human Jurkat T-lymphoma cell line as a test model to evaluate whether β-estradiol-induced cytotoxicity is associated with oxidative stress.

## 2. MATERIALS AND METHODS

### 2.1. Chemicals and Test Media

Growth medium RPMI 1640 containing 1 mmol/L L-glutamine was purchased from Gibco BRL products (Grand Island, NY). Fetal bovine serum (FBS), phosphate buffered saline (PBS) were obtained from Sigma Chemical Company (St. Louis, MO).

### 2.2. Tissue Culture

Human T-lymphoma (Jurkat) cells were from American Type Culture Collection (Manassas, VA, USA). In the laboratory, cells were stored in the liquid nitrogen until use. They were next thawed by gentle agitation of their containers (vials) for 2 minutes in a water bath at 37°C. After thawing, the content of each vial of cell was transferred to a 25 $cm^2$ tissue culture flask, diluted with up to 10 mL of RPMI 1640 containing 1 mmol/L L-glutamine (GIBCO/BRL, Gaithersburg, MD) and supplemented with 10% (v/v) fetal bovine serum (FBS), and 1% (w/v) penicillin/streptomycin. The 25 $cm^2$ culture flasks, each containing $2 \times 10^6$ viable cells, were observed under the microscope, followed by incubation in a humidified 5 % $CO_2$ incubator at 37°C. Three times a week, they were diluted under same conditions to maintain a density of $5 \times 10^5$/mL, and harvested in the exponential phase of growth.

### 2.3. Cell treatment and biochemical test for cell viability

The *in vitro* cytotoxicity of β-estradiol against Jurkat T-cells was determined by MTT assay as previously described (Yedjou and Tchounwou, 2007). Briefly, to 180 μL aliquots in six replicates of the cell suspension ($5 \times 10^5$ cells/mL) seeded to 96-well polystyrene tissue culture plates, 20 μL aliquots of stock solutions were added to each well using distilled water as solvent to make-up final β-estradiol concentrations of 1, 2, 4, 8, and 16 μM. Control cells received 20 μL of distilled water. Cells were placed in a humidified 5% $CO_2$ incubator for 12 hr at 37°C. After incubation, 20 μL aliquots of MTT solution (5 mg/mL in PBS) were added to each well and re-incubated for 4 h at 37°C following by low centrifugation at 800 rpm for 5 min. Then, the 200 μL of supernatant culture medium were carefully aspirated and 200 μL aliquots of dimethylsulfoxide (DMSO) were added to each well to dissolve the formazan crystals, followed by incubation for 10 min to dissolve air bubbles. The culture plate was placed on a Biotek micro-plate reader and the absorbance was measured at 550 nm. All assays were performed in six replicates for each β-estradiol dose. Statistical analysis was done to determine the means ± SDs of cell viability. Cell viability rate was calculated as the percentage of MTT absorption as follows: % survival = (mean experimental absorbance/ mean control absorbance×100).

### 2.4. Biochemical test for oxidative stress by lipid peroxidation assay

Lipid peroxidation is traditionally quantified to measure malondialdehyde (MDA) and 4-hydroxynonenal. The extraction procedure and measurement of the extracted MDA was

performed according to the manufacturer's instructions (Calbiochem-Novabiochem, San Diego, CA). Malondialdehydes (MDA) are formed during lipid peroxidation (Halliwell and Gutteridge 1984). Briefly, $2 \times 10^6$ Jurkat T-cells/mL untreated as a control and cells treated with β-estradiol were incubated in a total volume of 10 ml growth medium for 48 hours. After the incubation period, cells were collected in 15 mL tubes, followed by low-speed centrifugation. The cell pellets were re-suspended in 0.5 ml of Tris-HCl, pH 7.4, and lysed using a sonicator (W-220; Ultrasonic, Farmingdale, NY) under the conditions of duty cycle-25% and output control-40% for 5 sec on ice. A 200 μl aliquot of the culture medium was assayed for MDA according to the lipid peroxidation assay kit protocol. The absorbance of the sample was read at 586 nm, and the concentration of MDA was determined from a standard curve.

### 2.5. Statistical analysis

Experiments were performed at least in triplicates. Data were represented as means ± SD. Where appropriate, one-way ANOVA test or paired t-test was performed using SAS Software available in the Bio-statistics Core Laboratory at Jackson State University.

## 3. RESULTS

### 3.1. β-estradiol Inhibits Cellular viability of Jurkat T-lymphoma cells

We used the MTT [3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay to examine the cytotoxic effect of β-estradiol on Jurkat T-cells for 24 and 48 hours, respectively (Figure 2). Data generated from these studies clearly showed that β-estradiol exposure significantly reduces the viability of these cell lines. After 24 and 48 hours of exposure, β-estradiol exerted a significant cytotoxic effect on Jurkat T-cells in dose- and time-dependent, showing a response relationship with increasing doses of β-estradiol. We observed a biphasic response where physiological (low) doses of β-estradiol induce cell growth and cellular proliferation of Jurkat T-cells whereas higher doses inhibit cell growth and induce cell death.

### 3.2. β-estradiol Induces Oxidative Stress in Jurkat T-lymphoma cells

The standard curve generated from lipid peroxidation assay is presented in Figure 3, and the effect of β-estradiol on malondialdehyde (MDA) production in Jurkat T-cells is presented in Figure 4. MDA is an end product of lipid peroxidation which is a key determinant of cellular injury. As seen on figure 4, there is a significant increase in MDA level at all doses tested as result of oxidative stress, a biomarker of cellular injury. A maximum level (21.5 μM) of malondialdehyde (MDA) production was observed at the highest dose tested compared to (4.95 μM) in the control. This increase demonstrates that β-estradiol augments MDA production in Jurkat T-cells.

## 4. DISCUSSIONS

Natural estrogens such as estradiol, estrone, and estriol are produced from natural sources such as the urine of pregnant mares and women, the adrenals and testes of stallions, and the human placenta and amniotic fluid (Roberts, 1986). To prove the cytotoxic efficacy of β-

estradiol in Jurkat T-cell model, cell viability was determined by the MTT assay. As shown in Figure 2, β-estradiol treatment increased the percentage of dead cells in a dose- and time-dependent manner. Consistent with our results, *in vitro* studies indicated that the addition of estradiol to cultures of human lymphocytes causes a decrease in CD4+/CD8+ T-cell-subset ratios (Athreya et al., 1993), and enhances immunoglobulin secretion (Kanda and Tamaki, 1999). Several scientific reports indicated that estrogen toxicity resulted in the death of the patient (Suess et al., 1992; Sanpera et al., 2002) or in a long recovery period (Hall, 1992; Acke et al., 2003).

To test the hypothesis that oxidative stress may be involved in β-estradiol induced anti-tumor activity in Jurkat T-cells, we performed lipid peroxidation assay. Our result obtained from this assay showed a slight significant ($p < 0.05$) increase in malondialdehydes (a by-product of lipid peroxidation and biomarker of oxidative stress) production in β-estradiol-treated cells compared to the control. Similarly, a recent study in our laboratory indicated that garlic compounds generate MDA production in HL-60 cells leading to phosphatidylserine externalization, caspase-3 activation, nucleosomal DNA fragmentation, and cell death (Yedjou and Tchounwou, 2012). High level of MDA production detected in the Jurkat T-cells may be indicative of the therapeutic mechanisms of action of β-estradiol in human T-lymphoma (Jurkat) cells.

## 5. CONCLUSIONS

Results from this study indicate at the cellular level that β-estradiol significantly reduced the viability of human T-lymphoma (Jurkat) cells in a dose and time-dependent manner. At the molecular level, β-estradiol treatment resulted in a significant increase in lipid peroxidation generation in Jurkat T-cells. Findings from this study indicate that β-estradiol is highly cytotoxic to Jurkat T-cells. This cytotoxicity is found to be mediated through oxidative stress, a biomarker of cellular injury.

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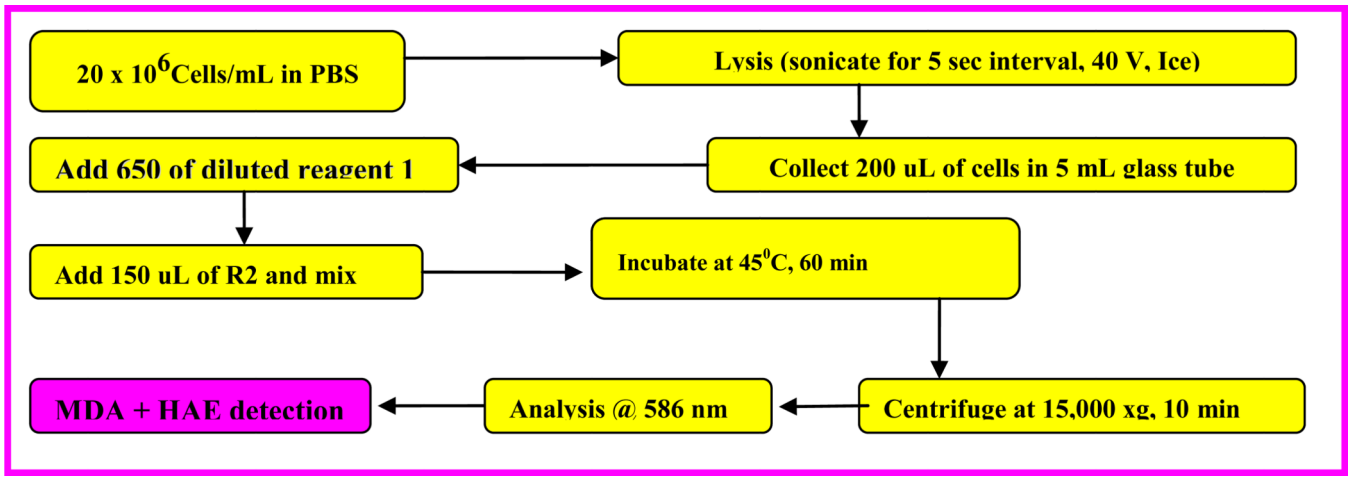


**Figure 1.**
Schematic representation of the steps in Lipid Peroxidation assay

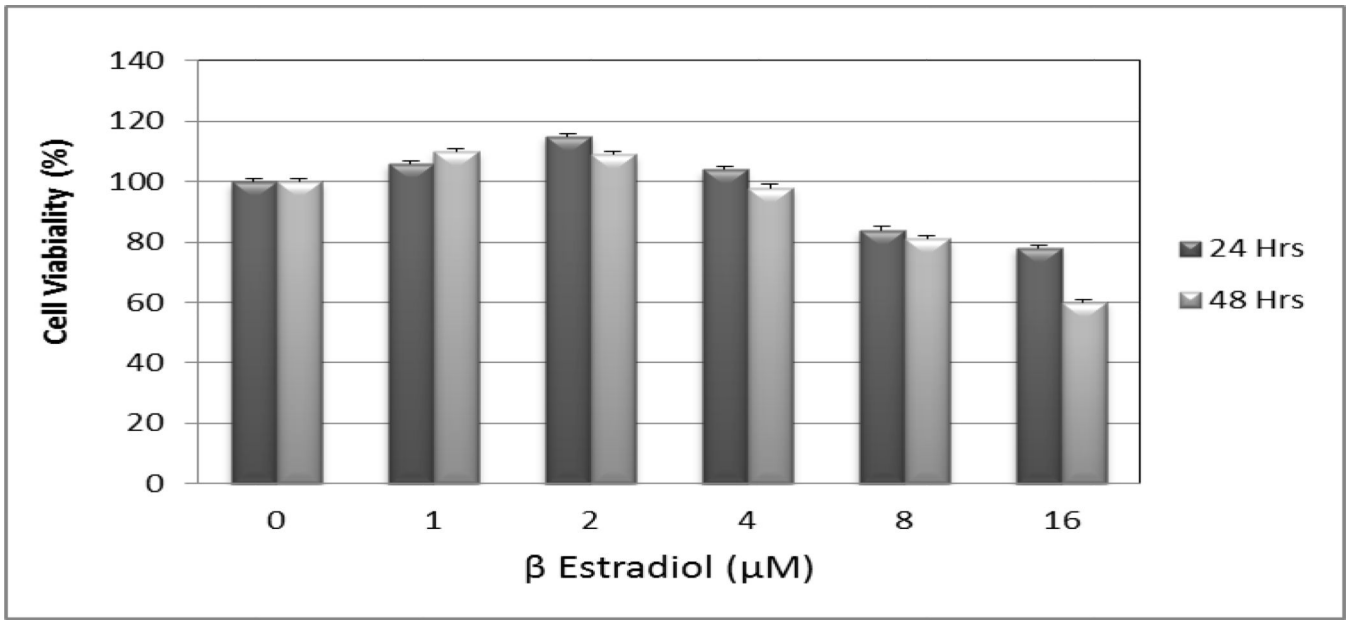


**Figure 2.**

β-estradiol potently induces toxicity to human T-lymphoma (Jurkat) cells. Jurkat T-cells were treated for 24 and 48 hours with medium supplemented with solvent and various doses (1–16 μM) of β-estradiol.

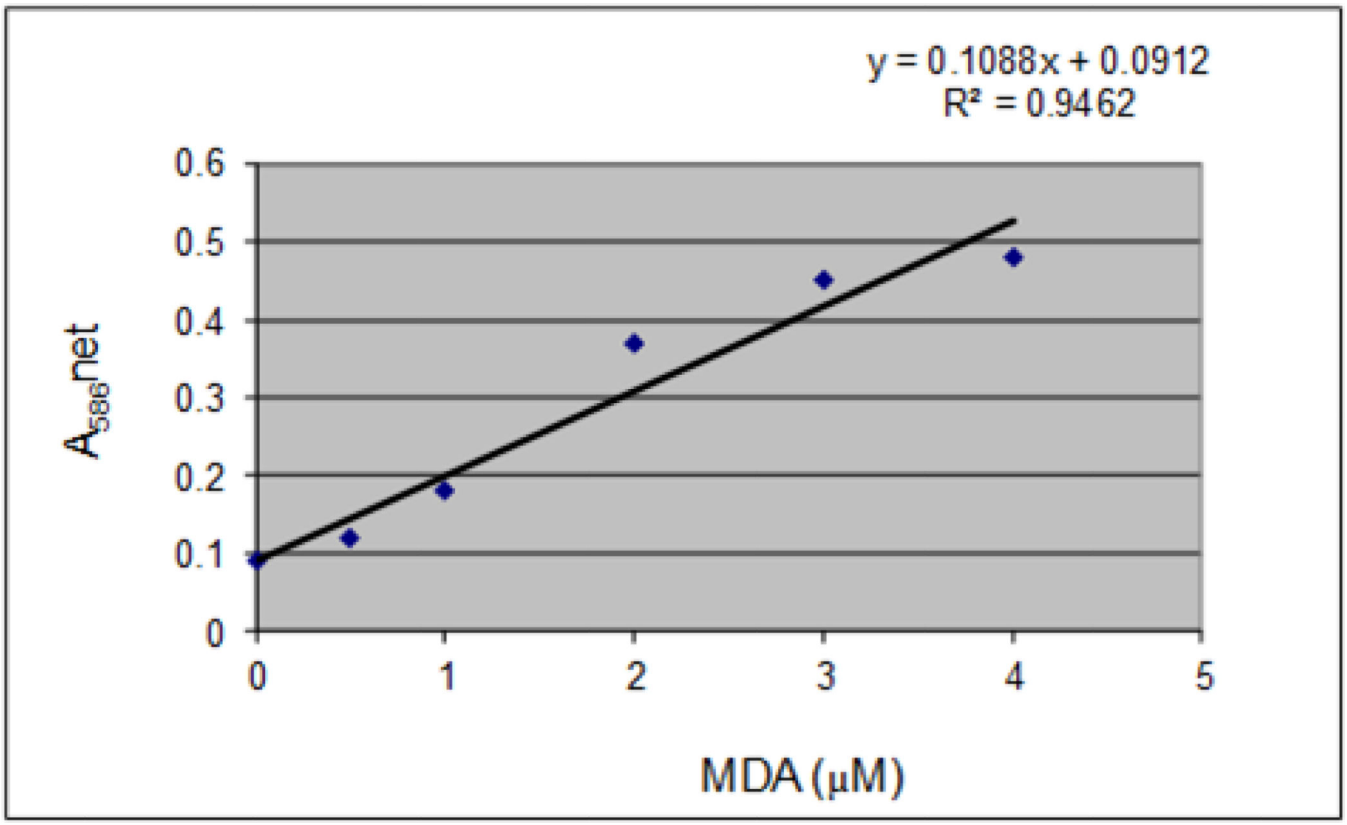


**Figure 3.**
MDA standard curve showing the net absorbance at 586 nm as a function of MDA concentration.

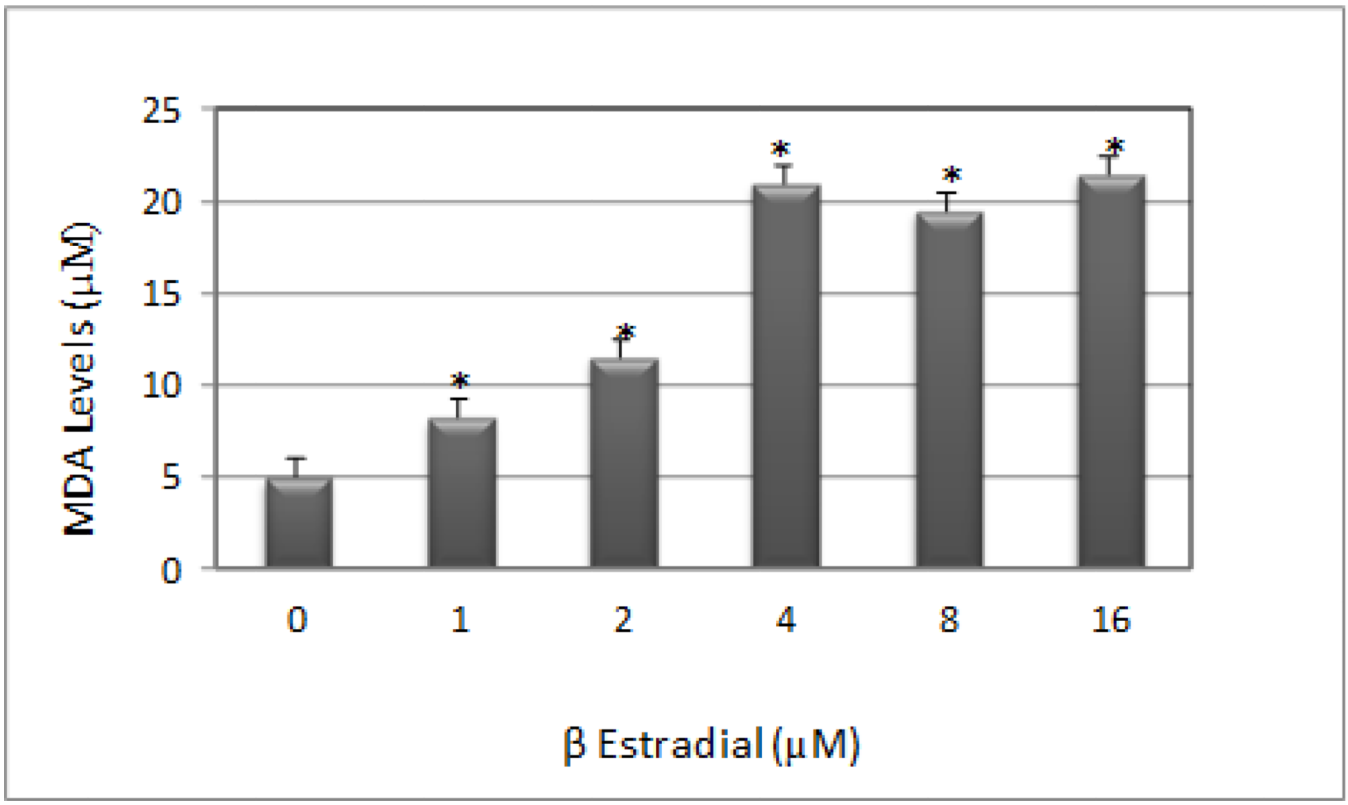


**Figure 4.**

β-estradiol-induced oxidative stress in human T-lymphoma (Jurkat) cells. Cells were incubated for 48 hours with increasing doses of β-estradiol (1, 2, 4, 8, and 16 μM). Malondialdehyde (MDA) concentrations were determined as described in Materials and Methods. *Significantly different from the control by ANOVA Dunnett's test; $P < 0.05$. Data are representative of 3 independent experiments.