Effect of Nickel Sulfate on Yeast Growth and Cell Division

Suliman M Hussein, Fauzia R El garabulli and Aml O Alhadad*

Botany Department, Faculty of Science, Benghazi University, Benghazi, Libya

*Corresponding Author: Aml O Alhadad, Botany Department, Faculty of Science, Benghazi University, Benghazi, Libya.

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Abstract

Environmental factors influence carcinogenesis by interfering with a variety of cellular targets. Quantitative evaluation of genetic effects of atmospheric pollutant; ozone, cigarettes smoke and metals, have been performed in yeast system by many workers. In this work, the effect of nickel sulfate on growth and cell division was investigated in yeast culture (*Saccharomyces cerevisiae*). The results revealed that, there are significant changes in the yeast dry weight and cell number in samples treated with different concentrations (0, 4, 10, 20, 100, 200 ppm) for different time (4, 10, 24, 48h). These changes were concentration and time dependent. In addition, there were changes in size and shape of yeast cells and in colony size.

Keywords: Nickel Sulfate; *Saccharomyces cerevisiae*; Dry Weight; Cell Division

Introduction

Nickel is a metal of widespread distribution in the environment. Contact with soluble and insoluble nickel compounds can cause a variety of side effects on human health. Human exposure to Ni may occur through food, water or air [1]. At higher concentrations, nickel ion can be very toxic to both eukaryotic and prokaryotic cells, it potentially inhibits synthesis of macromolecules such as RNA and protein [2,3]. The binding of nickel ions to cellular protein, followed by reaction with endogenous hydrogen peroxide formed by cellular metabolism, could lead to oxygen radical generation and chromosome breakage, which could cause mutation and activation of proto-oncogenes into activated oncogenes [4-7]. Nickel has been classified as a human carcinogen based on epidemiological evidence, which shows high incidence of nasal and lung cancers in refinery workers [8-11]. The results of many in vivo and in vitro researches suggest that nickel and nickel oxide nanoparticles are responsible for lung toxicity, inflammation, oxidative stress and apoptosis [12,13]. Although the toxicity and carcinogenicity of nickel compounds in humans and experimental animals are well demonstrated, the underlying mechanisms of their action

remain unclear [14,15]. The mutagenicity and recombinogenicity of environmental pollutant were investigated in yeast system [16-23]. *Saccharomyces cerevisiae*, commonly known as baker's yeast, has been a preferred organism for genetic research since the mid-twentieth century. The experimental value of this single-celled eukaryotic species lies in its simple life cycle, alternating haploid and diploid phases, short generation time, and easy-to-identify meiotic products. Recent technological advances enable yeast biologists to use genetic analysis to gain a deep understanding of the organization and regulation of eukaryotic cells [24].

The aim of the present work was to investigate the cytotoxic effects of nickel sulfate on cell division and dry weight using yeast as biological system.

Materials and Methods

Nickel sulfate (from BDH chemicals Ltd. Poole – England.) was chosen for the present investigation. Commercial yeast (*Saccharomyces cerevisiae*, S.I. Lesaffre-France) was grown in a complex medium (Malt Extract Broth, from OXOID Ltd., Basingstoke, Hamp-

shire, England), with pH adjusted to 5.2. The media were enriched with nickel sulfate, in such quantities that initial nickel sulfate concentrations were 4, 10, 20, 100 and 200 ppm. Sterile media (40 ml/treatment) was placed in conical flasks, inoculated with 1ml of pure yeast culture and left in a shaking incubator (30°C, 150 rpm) for different time exposure (4, 10, 24, 48 hours). Three replicates of each treatment were taken. Yeast growth was monitored by measuring the yeast cell number per ml using Haemocytometer (Improved Neubauer, R.S. 749. Supplied by Gallenkamp) as in [25]. The dry weight of yeast culture was measured as in Pasternakiewicz (2006). Then the data were statistically tested. The effect of Ni concentration in the medium on yeast growth was estimated using Two-way Analysis of Variance (ANOVA) which was calculated with SPSS computer package.

Results, Discussion and Conclusion

Microorganism show sigmoidal growth due to eventual limitations of space or nutrient accumulation of end product but the overall growth of any organism includes an early exponential phase in optimized normal media. Any changes in rate of growth had to deal with interfering of tested substances with bioactivity of organism or the cell. The rate of growth can be measured by many growth parameters such as volume, biomass (Dry weight), cell number and protein content [22,26-28]. The effect of different concentrations of nickel sulfate and also different time intervals on yeast cell number are represented in figure 1.

The results showed that there are significant differences within treatments ($P < 0.01$). Natural increase of yeast cell number in the control from 1.85×10^7 at 4h to 8.68×10^7 at 48h. Treated cells with 4ppm stimulate yeast cell division at 4hr exposure time (2.046×10^7). On other hand, there was no significant change observed in cell number in samples treated for 10, 24, 48hr (6.791 , 7.177 and 8.780×10^7 respectively). There were decrease in cell number in samples treated with high concentrations (100, 200ppm) in all exposure times. In meanwhile, 20ppm induce an increase in cell number at all exposure times. 10ppm cause increase in cell number in samples treated for 4hr and 48hr, slight decrease observed in 10, 24hr (7.058 , 8.815×10^7 respectively).

Over all, the effect of nickel sulfate on the cell number was increase by increase time of treatment and depend on nickel sulfate concentration $\{P(0.015)\}$ comparing to the control. There were highly significant mean differences at (0.05 level) between the effect of high concentrations (100 and 200 ppm) and the other used

concentrations ($P > 0.001$). All treatment times had significant effect on the cell number represented by decrease or increase in total cell number, which is an evidence of nickel sulfate genotoxicity [29].

The effects of different concentrations of nickel sulfate and different time intervals on yeast dry weight are represented in figure 2. The results showed that there are significant differences within treatments ($P < 0.01$). Low concentration of nickel sulfate (4, 10, 20 ppm) did not cause significant change in biomass of treated *Saccharomyces cerevisiae* cells comparing to the control, whereas high concentrations (100, 200 ppm) cause higher significant effect on dry weight of treated cells comparing to the control and low nickel sulfate concentrations.

Figure 1: Number of dividing cells in yeast culture treated with different NiSO₄ concentration for different time.

Figure 2: Dry weight of yeast culture treated with different NiSO₄ concentration for different time.

In the condition present in this experiment, the cell number strongly decreased with high concentration of nickel sulfate treatment (100 and 200 ppm). The achieved results suggested that the decrease in dry weight may result of inhibition of cell division. The observed data agree with the results of Dovgaliuk and others (2001) who found that nickel sulfate inhibition the root growth and reduce mitotic activity of merestimic cells [30]. Pasternakiewicz (2006) found out that the biomass yield of *Saccharomyces cerevisiae* was lower in medium enriched with cadmium and this action was concentration dependent [22]. Both the specific growth rate and the biomass concentration were more inhibited in the bioaccumulation media containing Ni(II) ions; where the increase of Ni(II) concentration led to a drastic decrease in microbial growth for *Candida utilis* [28]. The decrease in dry weight and cell division may result of the inhibition of protein synthesis [31].

Nickel sulfate caused cell and colony abnormalities represented by changes in their size and shape. The size of normal yeast cell in this experiment under optimized conditions were 8 to 14 μ depend on stage of cell division. Change in cell size was observed in culture treated with high concentrations of nickel sulfate to the size ranged between 7 to 10 μ , change in the shape of the cells were observed also at the same concentrations (Figure 3). Recultivation of treated cells on solid medium give abnormal size of yeast cells and colonies compare to the control with decrease in the colonies number at the same dilution, which is an evidence of the effect of nickel sulfate on cell division (Figure 4).

Cytoskeleton and cell protein could be a cell target for nickel sulfate [30,32,33]. Change of the protein structure cause a decrease in enzyme activity which lead to inhibition of cell growth or induction of mutation. The nickel binding to protein induced modification in its secondary structure [21] which lead to negative impact in cell growth as result of mutation. Nickel toxicity may lead to damage the membrane integrity result in aberrant cell [34,35].

The experiment showed the negative influence of nickel sulfate on the growth of *Saccharomyces cerevisiae*. Decrease in yeast cell number at high nickel concentrations and long time exposure indicated the inhibition of yeast cell reproduction. Higher nickel concentrations cause drastically decreased dry weight which suggested that nickel in high concentration in the media impaired the synthesis of protein which lead to the decrease of cell number and dry weight.

Figure 3: Changes in size and shape of cells in yeast cultures treated with high concentrations of nickel sulfate for long time exposure.

Figure 4: Abnormal size of yeast cells and colonies after re-inoculated of treated cells with different NiSO₄ concentrations for different exposure time.

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