# Protective efficacy of Andrographolide against Nickel induced Sister Chromatid Exchanges in Human Lymphocytes

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## *Abstract*

*Andrographolide, the main constituent of Andrographis paniculata is widely used as herbal remedy due to its anti-inflammatory, antioxidant and anti-cancer activities. In this study, antigenotoxic potential of andrographolide (AG) has been assessed by analyzing the DNA damage in the form of sister chromatid exchange (SCE) in cultured peripheral blood lymphocytes (PBLs).*

*Cells treated with nickel chloride (1 mM) in combination with different concentration andrographolide (4-20 µg/ml) showed significant ($p<0.05$) reduction in the formation of sister chromatid exchanges. The data suggests that andrographolide has strong antigenotoxic potential to inhibit sister chromatid exchanges induced by nickel.*

**Keywords**: Nickel, andrographolide, Sister chromatid exchange, Genotoxiciity, Antigenotoxicity.

## Introduction

Experimental evidences show that soluble nickel compounds such as nickel chloride are potent inducers of oxidative stress, DNA damage mediated by free radical formation[3]. Exposure to some nickel compounds has a strong correlation with respiratory cancer[5].

Naturally present bioactive compounds from plants have beneficial effects against diseases, genotoxicants and carcinogens. *Andrographis paniculata* is one of these plants and the main source of its bioactive compound is andrographolide (AG). Studies have shown that this bioactive compound has antiviral, antibacterial, anticancerous, anti-inflammatory and antipyretic properties[11,16].

Sister chromatid exchange (SCE) is a conserved process by which reciprocal exchange of DNA segments occurs by homologous recombination between sister chromatids during replication. It is an error-free as the sequence of DNA is generally not altered. A higher frequency of sister chromatid exchange is an indication of random chromosomal instability and used as an important biomarker of genotoxicity and carcinogenicity.

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SCEs can be induced by DNA damaging agents[21]. Hence, in this study we evaluated role of andrographolide against nickel induced SCEs in cultured peripheral blood lymphocytes.

## Material and Methods

Nickel chloride (CAS No. 7718-54-9) and andrographolide (CAS No. 5508-58-7) were obtained from Sigma Aldrich and other experimental and culture related chemicals, solutions and reagents were obtained from Himedia.

**Subject recruitment:** After taking consent, venous blood (4-5 ml) was taken from healthy participants (Age 25-30 years) in heparin-coated "Vacutainer Tubes" (Becton-Dickinson and Co.) under aseptic conditions. Ethical clearance for this research was given by the Institutional Human Ethics Committee, Kurukshetra University, Kurukshetra vide letter No. IHEC/17/421.

**Culture set up:** Blood culture was set up for 72 hours according to Moorhead et al[10] with slight modifications. In each glass culture tube, RPMI 1640 (5 ml), FBS (20%), penicillin (100 UI/ml) and streptomycin (100 µg/ml) and PHA (2%) and 400 µl of collected blood were added and incubated for 72 hours (3 cell cycles) at 37 ºC and ±5% $CO_2$ and 95% humidity. Andrographolide treatment at different concentrations (4-20 µg/ml) was given to cells treated with 1 mM nickel to evaluate its protective role against nickel. Tubes were gently stirred in between the incubation period to allow proper mixing of contents, aeration and prevention of cell aggregation.

**Sister chromatid exchange assay:** The assay was conducted according to method of Wolff and Perry 1974[22] with slight modifications. After 24 hours, 5-Bromo-2-deoxyuridine was added at a concentration of 10 µg/ml of the culture. To arrest the cells at metaphase, 2-3 drops of colchicine (0.2 µg/ml) were added to the culture 45 minutes before harvesting.

Cells were centrifuged and gradually resuspended in 0.075 M KCl at 37º C for 10 minutes to give hypotonic treatment. After hypotonic treatment, chilled fixative (3:1; methanol: glacial acetic acid) was mixed with cells to fix them.

Slide-preparation was done by letting small drops of the cells suspension fall from a height around 4ft on clear glass slides and dried to follow the next step. Slides were put in Hoechst

33258 stain 20 µg/ml solution for 45 minutes in the dark. SSC (Sodium Saline Citrate) buffer was layered on in slide and covered with glass cover. UV exposure was given to slides for 2 hours before final staining with 4% Giemsa stain. Fifty metaphase cells were analyzed for scoring the SCEs.

**Statistical analysis:** Statistical analysis was done using SPSS software. The significance of the differences between means of control and the treated group were analyzed using Student's *t*-test. Mean values and standard deviations were computed and the calculation of statistical significance (P < 0.05) was done using One way Analysis of Variance (ANOVA).

## Results and Discussion

Cells were treated with different nickel chloride concentrations ranging from 0.125 mM to 1 mM (figure 1). With an increasing dose of nickel, the frequency of SCEs kept increasing. It can be seen that at a concentration of 1 mM, highly significant increase in SCE frequency was observed with mean 9.2±0.44.

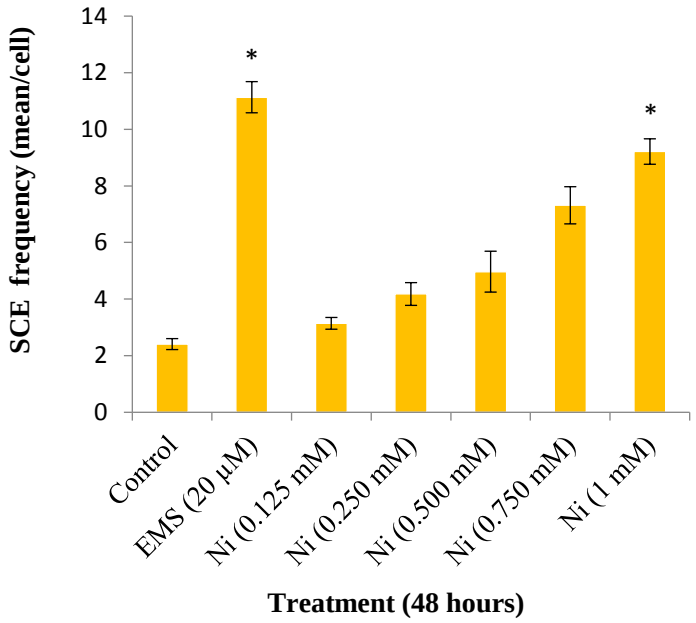


**Figure 1: Frequency Mean SCE /cell in cultured PBLs treated with different concentrations of nickel (0.125- 1 mM). * p<0.05 significant versus control**

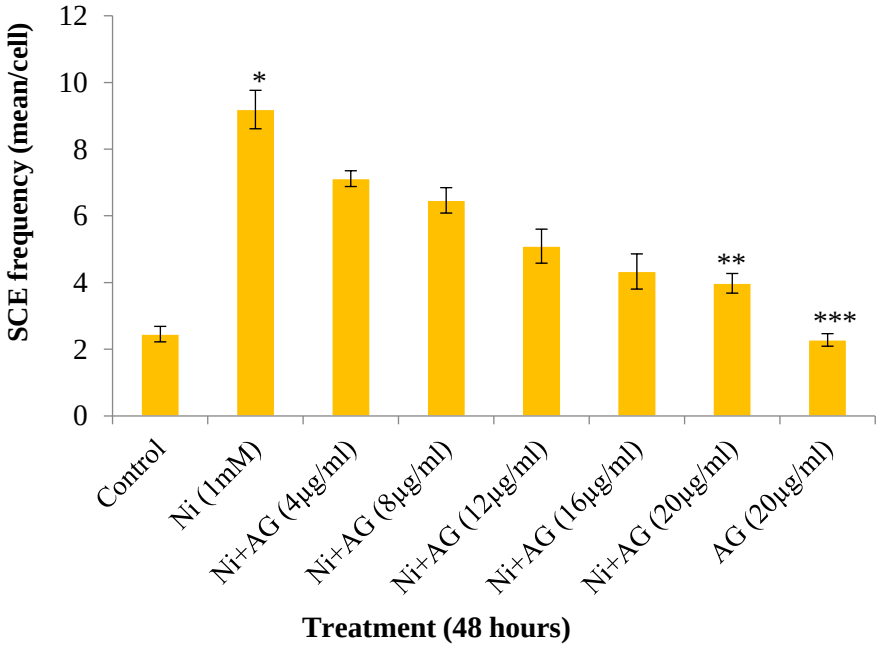


**Figure 2: Antigenotoxic effect of different concentrations of andrographolide (4-20 µg/ml) on SCE frequency in nickel (1 mM) treated PBLs. * p<0.05 significant versus control, ** p<0.05 significant versus treated and *** p>0.05 non-significant versus control**

Antigenotoxic efficacy of andrographolide was checked by treating cells with 4-20 µg/ml of Andrographolide for 48 hours (around two rounds of cell division) along with 1 mM nickel (figure 2). It was observed that andrographolide reduced the incidence of SCEs in a dose dependent manner. Andrographolide treatment significantly decreased the frequency of SCEs (mean/cell 3.97±0.29) at a dose of 20µg/ml as compared to cells treated with nickel only.

The overall results for the different samples (n=30) have been represented in figure 3. The results showed that andrographolide significantly reduced the incidence of SCEs at the selected dose against 1 mM nickel. Cells treated alone with andrographolide 20 µg/ml resulted in no significant difference as compared to control and therefore, it is found to be non-genotoxic to the lymphocytes at this dose. Representative images of SCE in control, nickel treated and andrographolide have been shown in figure 4.

Nickel chloride treatment results in a significant elevation in the frequency of SCEs/cell which is in agreement with the other reports in human lymphocytes[9,14,15]. It was reported that the oxidative stress induced by nickel compounds leads to a variety of genetic defects on chromatin[4]. Ni and its compounds cause oxidative stress by direct ROS generation, suppression of antioxidative system and lipid peroxidation which is the main cause of DNA damage[6].

The reduction in the SCEs upon andrographolide treatment is in concord with many studies[1,2]. Andrographolide can reduce $H_2O_2$ formation in human neutrophils and thus regulated ROS[18]. Andrographolide has anticancerous activity against lung cancer cells H3255 by downregulating PKC activity, suppressing TGF-β1 and VEGF expression and reducing $Na^+$-$K^+$-ATPase activity[8].

It has been reported that the aquous extract of *Andrographis paniculata* increases the antioxidant enzyme activities such as superoxide dismutase, catalase and glutathione S transferase[20]. Studies have reported that andrographolide has ROS scavenging activity which reduced the ROS level in cells[13,19,23].

Another study found that the andrographolide has a favorable structure for allylic hydrogen transfer on its c-11 position and acts as a chain-breaking antioxidant[7]. The selected concentration of andrographolide did not show genotoxicity in cells. This is in close relation with a study in which andrographolide did not show genotoxicity but induced dose-dependent necrosis at higher doses in AHH-1 and MCL-5 cells[17].

It has also been reported that orally administered andrographolide is quickly absorbed in blood to maximum plasma levels after 1.5–2 hours of oral administration.This bioavailability of andrographolide adds to its effectiveness as a drug[12]. Based on the above findings and our results, it is observed that free radical scavenging and hydrogen transfer may be the mode of antioxidant activity of andrographolide against nickel chloride in our study.

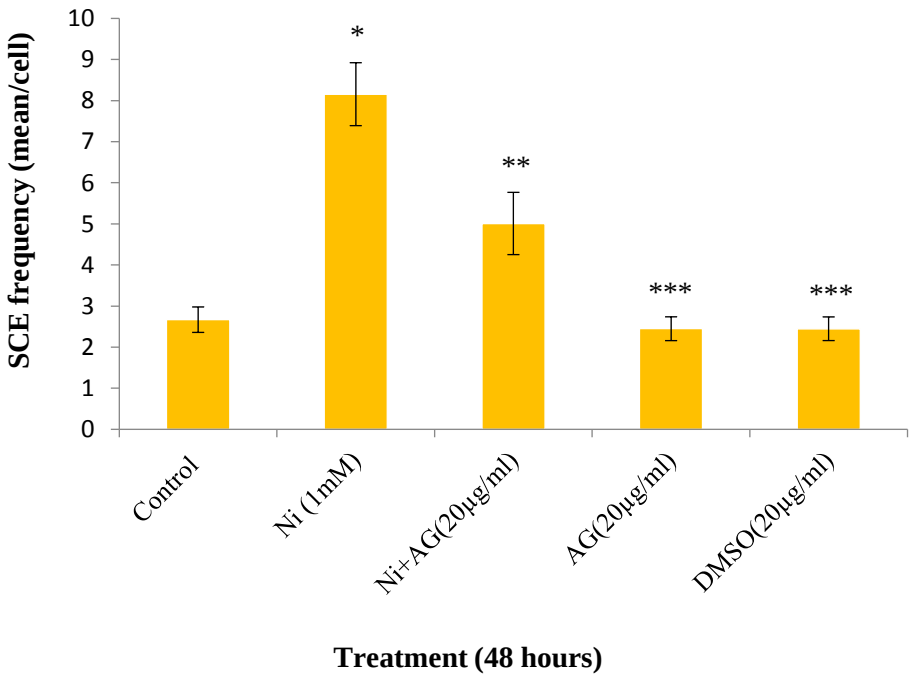


**Figure 3: Antigenotoxic effect of andrographolide (20 µg/ml) on SCE frequency in nickel (1 mM) treated PBLs in different samples (n=30). * p<0.05 significant versus control, ** p<0.05 significant versus treated and *** p>0.05 non-significant versus control**

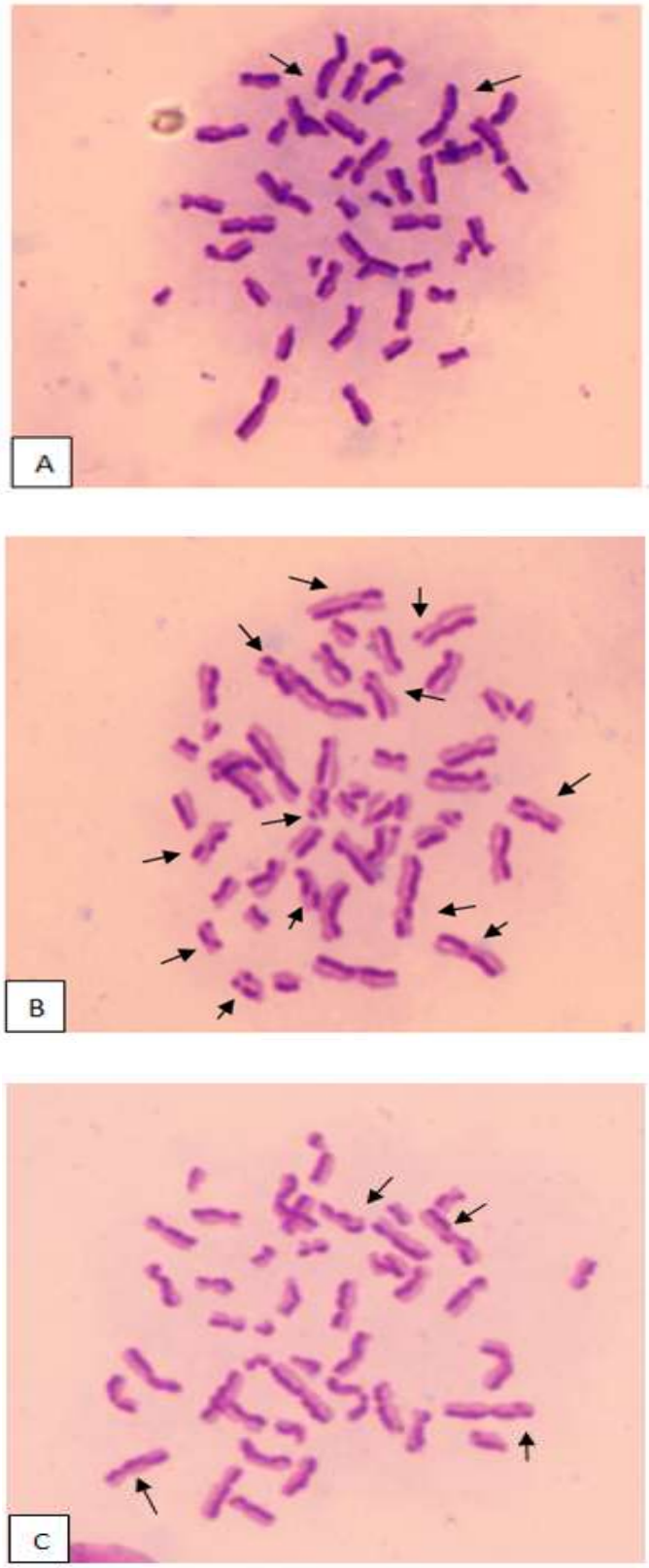


**Figure 4: Sister chromatid exchanges in PBLs. A) Untreated cells, B) Nickel treated cells and C) Andrographolide +Nickel treated cells**

## Conclusion

Andrographolide significantly reduced the sister chromatid exchanges against nickel and may be used as an active component in antioxidative formulations.

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