Evaluation of curcumin as potential herbal agent for mitigation of nickel and chromium induced micronuclei in human blood cultures

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Abstract
Chromium and nickel are widely used industrial chemicals. Welders in India are inclined to possible occupational chromium and nickel exposure. We have investigated the Genotoxicity exerted by nickel (Ni) in the form of nickel chloride (4.216 X 10^{-5}M) and chromium (Cr) in the form of potassium dichromate (1.36 X 10^{-6}M), alone and in combination and mitigating effect of curcumin (3.87 X 10^{-7}M) was studied at two different exposure intervals (24 and 69 hours) in toxicant added human blood cultures. Evaluation of genotoxicity was done by micronuclei (MN) assay. One thousand binucleate cells per group were analyzed. Statistical analysis was performed by one way ANOVA followed by Dunett’s test and percentage amelioration was calculated. Nickel and chromium proved to be more toxic in 69-hours exposure than in 24 hours in induction of micronuclei. Curcumin supplementation exhibited significant (71%-92%) amelioration at both exposure intervals. Hence, curcumin has a mitigating effect against micronuclei induced by metal salts due to its antioxidant and free radical scavenging properties.

Key words: Nickel chloride, Potassium dichromate, Curcumin, Genotoxicity, Micronuclei Human blood cultures.

Introduction
Chromium (Cr) and nickel (Ni) are naturally occurring elements and present in several different forms in the environment. The general population is exposed to chromium by inhaling ambient air, ingesting food and drinking water containing chromium or by direct skin contact. Occupational exposure to workers in industries related with pigment and chromate production, leather tanning, stainless steel welding, chrome plating etc. (1). Chromium (VI) is an environmentally known human carcinogen with complicated metabolism and an unknown mechanism of mutagenesis (5, 27). Chromium (VI) causes mutation and allied effect such as chromosomal aberrations in a wide range of prokaryotic and eukaryotic test systems, both in vivo and in vitro. The majority of studies reported genotoxic effects of chromium (VI) in mammalian cells in vitro (10). It is documented that the blood from exposed workers contained higher levels of chromium, when compared with those obtained in the control group and that a significant increase in the frequency of micronuclei (MN) and the number of binucleate cells carrying MN (BNMN) were detected(32).

Nickel (Ni) is an abundant trace element. Air pollution, cigarette smoke, modern food processing equipment using nickel alloys and nickel-chromium dental casting alloys all are sources of nickel exposure to the general population (19). Ni (II) is a known mutagen in vitro and a carcinogen in rat primary hepatocyte cultures (27). Its compounds are also reported to have embryotoxic and genotoxic effects.
prenatally (13) as they cross the fetomaternal barrier and enter the fetus. These are easily taken up by the cells and react with protein, RNA and possibly DNA (23). From our laboratory it is reported that Ni salts induced genotoxic effect in blood cultures (19). These genotoxic indices were increased in combination of nickel and chromium added cultures (24,8).

Turmeric (T) is a yellow powder, derived from the rhizome of the plant *Curcuma longa* (*Zingiberaceae*) (28). Turmeric consists of a water-soluble component, turmerin and lipid-soluble component curcumin. Curcumin (diferuloylmethane), the yellow pigments in turmeric is widely used as a food coloring (curry) and preservative. In particular, it inhibits purified human immunodeficiency virus type 1 (HIV-1) integrase (14). It has also been consumed in amounts up to one g/day for thousands of years in countries such as India. Pharmacological studies have demonstrated its anti-tumor, anti-inflammatory, anti-infectious and antioxidant activity with very low toxicity (25,30). It is also noticed that oral administration of natural antioxidant curcumin at 400µM significantly inhibited induction of micronuclei and chromosomal aberrations produced by whole body exposure of α-radiation in mice (31). Data on role of curcumin on MN induction by Ni and Cr and their combination in blood cultures are limited and hence this study was undertaken.

**Materials and Methods**

**Subjects:** Venous blood was collected from healthy non-smoking individual, aging from 20 to 25 years old, with their consent, in sterile heparinised syringes. Detailed information regarding the pre-exposure of individual to any kind of drugs or habitual insult was taken.

**Peripheral blood lymphocyte culture (PBLC)**

Cultures were set-up for each individual according to the standard protocol of Perry and Wolff (1974). Seven ml of RPMI –1640 (Himedia, Mumbai, pH 7.4) already supplemented with fetal calf serum (FCS) 7% (Himedia, Mumbai), antibiotics (benzyl penicillin; Sarabhai Piramal Pharmaceuticals Ltd., Vadodara and streptomycin; Sarabhai Piramal Pharmaceuticals Ltd., Vadodara.) and 100-µl phytohemagglutin (PHA) (5mg/5ml distilled water; Sigma – Aldrich, USA), 0.5ml of blood were added. Toxicants were added at 0 hour and at 45th hour of incubation so as to expose the culture for 69 hours and 24 hours respectively.

Binucleate induction and micronucleus analysis was done following the method of Fenech (2000) with slight modifications. At the 69th hr., 30 µL of cytochalasin B (1mg/ml distilled water; Sigma – Aldrich, USA) was added for 24 hour allowing the cells to undergo karyokinesis but preventing cytokinesis, resulting into binucleate cells. At 93rd hour culture tubes were subjected to centrifugation at 2000 RPM for 15 minutes. The pellet obtained after centrifugation was treated with hypotonic solution (0.075 M KCl) (Merck, Germany) for 20 min at 37°C. These cells were then fixed by 1:3 acetone: methanol (acetomethanol) fixative and gently flushed. The culture tubes were then kept in an ice bath for 30 minutes.

Slides were prepared from the cell suspension obtained after two washes with fixative. These slides were then stained with 2% Geimsa stain (Himedia, Mumbai) and observed under the microscope at 45X for scoring micronuclei in accordance with binucleates.

**Groups studied:** Cultures were divided into eight groups. Group I was test control. While in Group II curcumin (Himedia, Mumbai) alone was added (10µl/7ml media, 3.87 X 10^{-7}M). Group III and Group IV were treated with Ni (NiCl₂; 4.216 X 10^{-5}M) (Himedia, Mumbai) and Cr (K₂Cr₂O₇; 1.36 X 10^{-4}M) (Himedia, Mumbai) respectively.
and Group V with a combination of the same concentrations of toxicants. Groups VI, VII and VIII were curcumin supplemented with Ni, Cr and Ni + Cr. Group IX was positive control ethyl methyl sulphonate (EMS; 1.93mM) (Merck, Germany) was also done. Exposure intervals (24 and 69 hours.) remained same for all the test materials.

**Analysis of parameters:** One thousand binucleates per group were analysed under the microscope for micronuclei occurrence. Percentage of amelioration was calculated by using the following formula (20):

\[
\frac{(\text{Pro-oxidant Group} - \text{Respective Antioxidant Group})}{(\text{Pro-oxidant Group} – \text{Control})} \times 100
\]

**Statistical analysis:** Results are expressed as mean ± SE. All the treated groups were compared with the control group, and the curcumin-supplemented groups were compared with their respective pro-oxidant groups by one way ANOVA followed by Dunett’s test. P values less than 0.05 were considered to be significant.

**Results**

Cultures treated with nickel and chromium alone for 24 hours of exposure (Group III and IV respectively), showed an increase in micronuclei as compared to the control, while combination of these metals (Group V) exerted significant (P<0.05) increase in frequency of micronuclei (Table 1). In the other set of experiment, cultures were treated with same doses of nickel and chromium alone and in combination (Group III, IV, V) for an exposure of 69 hours. Results revealed a significant (P<0.001) increase in micronuclei induction (Table 2). Curcumin co-cultures to 24 hr. exposure (Group II; Table 1) indicated 70.94%, 75.00 %, and 81.27 % of amelioration with nickel, chromium and nickel + chromium respectively. Similarly, curcumin had 92.08 %, 83.33% and 81.66 % of amelioration with same culture groups exposed for 69 hrs (Tables 1 and 2). Positive control ethyl methyl sulphonate (EMS) also showed significant induction of micronucleus in both exposure intervals. Figure 1 indicates the binucleate, Figure 2 indicates the binucleate with micronucleus and Figure 3 represents a binucleate with more than one micronucleus as a result of toxic effect of positive control EMS.
Figure 3: Bimucleate with more than one Micronucleus as a result of toxic effect of positive control

Table 1. Effect of curcumin on Ni- and/or Cr-induced micronuclei per 1000 binucleates in Human lymphocytes after 24-hr exposure (n=6)

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ± SE</th>
<th>Value in Percentage</th>
<th>P Value when compared to control</th>
<th>Amelioration %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>8.66 ± 0.99</td>
<td>100</td>
<td>0.00</td>
<td>Nil</td>
</tr>
<tr>
<td>Group II</td>
<td>8.50 ± 0.96</td>
<td>98</td>
<td>0.121 ns</td>
<td>Nil</td>
</tr>
<tr>
<td>Group III</td>
<td>9.83 ± 0.48</td>
<td>113</td>
<td>1.062 ns</td>
<td>Nil</td>
</tr>
<tr>
<td>Group IV</td>
<td>10.66 ± 0.84</td>
<td>123</td>
<td>1.538 ns</td>
<td>Nil</td>
</tr>
<tr>
<td>Group V</td>
<td>11.33 ± 0.49</td>
<td>131</td>
<td>2.412*</td>
<td>Nil</td>
</tr>
<tr>
<td>Group VI</td>
<td>09.00 ± 0.86</td>
<td>104</td>
<td>0.254 ns</td>
<td>70.94</td>
</tr>
<tr>
<td>Group VII</td>
<td>09.16 ± 0.87</td>
<td>106</td>
<td>0.379 ns</td>
<td>75.00</td>
</tr>
<tr>
<td>Group VIII</td>
<td>09.16 ± 0.54</td>
<td>106</td>
<td>0.445 ns</td>
<td>81.27</td>
</tr>
<tr>
<td>Group IX</td>
<td>14.00 ± 0.58</td>
<td>161</td>
<td>4.658***</td>
<td>0.00</td>
</tr>
</tbody>
</table>

***p<0.001, *p<0.05, ns = non significant
Genotoxic effect of Ni and Cr were studied in human blood cultures with respect to micronuclei test and mitigating effect of curcumin. It has been reported that heavy metals are cytotoxic and genotoxic in different eukaryotic systems in vivo and in vitro (2,6,18,22). In our study, blood cultures treated with nickel chloride and potassium dichromate for 24 and 69 hours exposures brought about an increase in frequency of micronuclei induction. This induction was significant in combination of metals. Further, chromium alone proved to be more toxic in induction of MN than nickel at both exposure intervals. Milosevic et al., (15) presented that the newborns exposed to environmental pollutants containing Ni and Cr illustrated high frequency of micronuclei, which supports our finding.

It is also known that nickel (II) and chromium (III) enter the cell directly while Cr (VI) penetrates only by means of sulfate ion channel. (11). These Ni (II) and Cr (III) ions induce the formation of the free radicals which cause lipid peroxidation as well as DNA damage. These free radicals also inhibit DNA repair enzymes followed by cell cycle regulatory enzymes leading to low cell proliferation. Five out of six workers working in chromium plating factories had a significant elevated unscheduled DNA synthesis (UDS) in pleural mesothelial cells (17). Antioxidant enzymes activity like superoxide dismutase(SOD) and lipid peroxidation levels also significantly altered in the pro oxidant treated groups indicating cellular damage (21). This would also support antioxidant damage at DNA levels in our report.

Chromium and nickel compounds further interact with DNA repair processes that lead to an enhancement of genotoxicity in combination with a variety of DNA damaging agents (9,12). Nickel induced

**Table 2.** Effect of curcumin on Ni- and/or Cr-induced micronuclei per 1000 binucleates in Human lymphocytes after 69-hr exposure (n=6)

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ± SE</th>
<th>Value in Percentage</th>
<th>P Value when compared to control</th>
<th>Amelioration %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>9.50 ± 0.34</td>
<td>100</td>
<td>0</td>
<td>Nil</td>
</tr>
<tr>
<td>Group II</td>
<td>9.50 ± 0.43</td>
<td>100</td>
<td>0</td>
<td>Nil</td>
</tr>
<tr>
<td>Group III</td>
<td>13.67 ± 0.42</td>
<td>144</td>
<td>7.679***</td>
<td>Nil</td>
</tr>
<tr>
<td>Group IV</td>
<td>15.50 ± 0.56</td>
<td>163</td>
<td>9.115***</td>
<td>Nil</td>
</tr>
<tr>
<td>Group V</td>
<td>17.60 ± 0.49</td>
<td>186</td>
<td>13.590***</td>
<td>Nil</td>
</tr>
<tr>
<td>Group VI</td>
<td>9.83 ± 0.40</td>
<td>103</td>
<td>0.632ns</td>
<td>92.08</td>
</tr>
<tr>
<td>Group VII</td>
<td>10.50 ± 0.43</td>
<td>110</td>
<td>1.826ns</td>
<td>83.33</td>
</tr>
<tr>
<td>Group VIII</td>
<td>11.00 ± 0.38</td>
<td>116</td>
<td>2.910*</td>
<td>81.66</td>
</tr>
<tr>
<td>Group IX</td>
<td>21.8 ± 0.74</td>
<td>229</td>
<td>15.083***</td>
<td>0.00</td>
</tr>
</tbody>
</table>

*\*p<0.001, \*p<0.05, ns = non significant
genotoxicity is also probably by the nickel catalyzed free radicals (3). Thus both metal ions are potential in generation of MN induction in our study.

Curcumin supplementation brought about protective effect on nickel and chromium induced micronuclei. It possesses parahydroxyl groups, which have antioxidant activity while other reactive groups like keto and double bonds of curcumin have anti mutagenic effects. Hence this herbal product scavenges and neutralizes free radicals generated during toxic reactions in response to the chemical insult, by breaking their subsequent oxidative chain reactions (4). The chelation effect of curcumin might also be suggestive that curcumin could be a potential candidate in removal of metal ions as suggested by Shen (26) for copper ions in Alzheimer’s disease. These effect needs to be further investigated for further metal ions. Thus, in our study, curcumin exhibits mitigating effect on nickel and chromium generated MN and is of value as one of the food supplements to occupationally exposed population to heavy metals.

Conclusion

From this study, we conclude that the increased exposure of cells to the nickel and chromium leads to DNA damage and curcumin had the potential mitigating effect on nickel and chromium exerted genotoxicity in vitro.

Acknowledgement

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References


