Research in Pharmaceutical Sciences, April 2024; 19(2): 178-187
Received: 06-06-2023
Peer Reviewed: 01-07-2023
Revised: 27-08-2023
Accepted: 22-01-2024
Published: 01-04-2024

School of Pharmacy & Pharmaceutical Sciences
Isfahan University of Medical Sciences

*Original Article*

# *In vitro* and *in vivo* assessment of indomethacin-induced genotoxicity: protection by curcumin

**Ehsan Zamani[1], Reza Alipour Klour[1,2], Amirreza Gholami Shekarsarayi[2,3], Foad Ghazizadeh[1], and Mehdi Evazalipour[3]**

[1]*Department of Pharmacology and Toxicology, School of Pharmacy, Guilan University of Medical Sciences, Rasht, Iran.*
[2]*Student Research Committee, School of Pharmacy, Guilan University of Medical Sciences, Rasht, Iran.*
[3]*Department of Pharmaceutical Biotechnology, School of Pharmacy, Guilan University of Medical Sciences, Rasht, Iran.*

## *Abstract*

**Background and purpose:** Indomethacin is one of the most widely used non-steroidal anti-inflammatory drugs. This study aimed to investigate the protective effects of curcumin against indomethacin-induced genotoxicity.
**Experimental approach:** For *in vitro* studies, human peripheral blood lymphocytes were obtained from a healthy volunteer and treated for 24 h as follows: vehicle control, indomethacin at 100 and 200 µM, indomethacin (100 µM and 200 µM) plus curcumin (27 µM). For *in vivo* experiments, mice received a single *i.p* dose of curcumin (100 mg/kg) and after 30 min genotoxicity induction was carried out by a single *i.p* injection of indomethacin at 10, 20, and 40 mg/kg. After 24 h, bone marrow cells were obtained from mice femurs. Genotoxicity was evaluated using a micronucleus assay. Oxidative damage was also inspected both *in vitro* and *in vivo*.
**Findings/Results:** *In-vitro* studies indicated that co-treatment with curcumin caused a significant decrease in the average micronuclei percentage and MDA level, and a significant increase in GSH concentration compared to the groups treated only with indomethacin. *In-vivo* findings revealed that pretreatment with curcumin induced a significant increase in the average ratio of polychromatic erythrocyte/normochromic erythrocyte, GSH concentration and caused a significant decrease in the average percentage of micronuclei and MDA level, in comparison with the group treated only with indomethacin.
**Conclusion and implications:** Curcumin attenuated indomethacin-induced genotoxicity both *in vitro* and *in vivo*. These effects might be partially exerted by decreasing oxidative stress. Further studies are required to elucidate the exact genoprotective mechanism of curcumin against indomethacin.

**Keywords:** Curcumin; Genotoxicity; Indomethacin; Oxidative stress.

## INTRODUCTION

Indomethacin is one of the most widely used non-steroidal anti-inflammatory drugs (NSAIDs) due to its potent anti-inflammatory and analgesic properties (1). However, indomethacin's adverse effects limit its use in clinical practice. Indomethacin, in addition to digestive tract ulcers, has other detrimental effects such as liver damage, kidney failure, blood abnormalities, and an increased risk of heart stroke (2). Although the pathophysiology of these complications is mainly attributed to the effect of indomethacin on cyclooxygenase and the subsequent lack of prostaglandins (2), other factors such as oxidative stress are also thought to be involved in the pathogenesis of indomethacin-induced damage (3).

*Corresponding author: M. Evazalipour
Tel: +98-1333486470, Fax: +98-1333486475
Email: evazalipour@yahoo.com

**Access this article online**

**Website:** http://rps.mui.ac.ir

**DOI:** 10.4103/RPS.RPS_100_23

Oxidative stress occurs when the balance between reactive oxygen species (ROS) production and antioxidant depletion is disrupted (4). High amounts of free radicals, including ROS, can cause genotoxic stress, which can lead to DNA damage and mutation (5). The mice bone marrow micronucleus (MN) assay is a popular *in vivo* test for the assessment of genotoxicity and DNA damage. The ratio of polychromatic erythrocyte (PCE) to normochromic erythrocyte (NCE) in bone marrow preparations is useful for screening chemicals with chromosome-breaking effects (6). Studies provide convincing evidence that indomethacin can stimulate the adhesion of leukocytes (primarily neutrophils) to vascular endothelium. Neutrophils can play a role in wound formation through the release of tissue-damaging proteases and reactive oxygen metabolites. This process is important in the pathogenesis of gastric ulcers process (7). Moreover, indomethacin can cause ROS production, and ROS formation plays an important role in the specific and idiosyncratic toxicity of indomethacin, such as bone marrow toxicity or liver inflammation (8). In addition, researchers have shown that indomethacin increases the concentration of malondialdehyde (MDA), an indicator of lipid peroxidation, by suppressing the antioxidant effect of ascorbic acid. Lipid peroxidation is known to be a common mechanism for the toxicity of many drugs (9).

Curcumin (1, 7-bis (4-hydroxy-3-methoxy-phenyl)-1, 6heptadiene-3, 5-dione,) is the main active ingredient of the *Curcuma longa* rhizome extract (10). It has little intrinsic toxicity but exhibits a wide range of pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, and anticancer properties (10). It has been speculated that the antioxidant and free radical scavenging activity of curcumin originates from the phenolic OH groups or the $CH_2$ group of the beta-diketone group of the molecule. Free radicals become neutral and inactive by receiving a proton from curcumin or by taking an electron (11). Furthermore, other studies also show that curcumin has a significant effect on all investigated parameters of oxidative stress, including reducing the level of MDA, increasing tissue activities of catalase (CAT), increasing superoxide dismutase (SOD) glutathione peroxidase (GPx) enzymes (12,13).

This study was undertaken to investigate the protective effect of curcumin on the prevention of indomethacin-associated genotoxicity both *in vitro* (in peripheral blood lymphocytes) and *in vivo* (bone marrow cells). The results of this study might help to a better understanding of the mechanism of indomethacin-induced genotoxicity and also provide a safe solution to attenuate indomethacin side effects.

## MATERIALS AND METHODS

### *Drugs and chemicals*

Indomethacin was purchased from Caspian Tamin Co. (Tehran, Iran), and curcumin (product No. C1386) was obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Cisplatin was obtained from Sobhan Daru (Tehran, Iran). All chemical compounds and reagents used in this study were of analytical grade (purity higher than 95%). Cytochalasin B was obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Dulbecco's modified eagle medium (DMEM), antibiotic-antimycotic, and trypsin-ethylenediaminetetraacetic acid (EDTA) were supplied from Bioidea Co. (Tehran, Iran). Phytohemagglutinin (PHA) and fetal bovine serum (FBS) were purchased from Gibco (Life Technologies, USA). Giemsa stain and dimethyl sulfoxide (DMSO) were purchased from Merck (Germany).

### *Genotoxicity evaluation*

*In vitro assessment of genotoxicity by MN assay in human peripheral blood lymphocytes*

The heparinized blood sample was obtained from the donor. The donor was a healthy, young, nonalcoholic, nonsmoking male. The donor had not been exposed to any chemicals or ionizing radiation that might have interfered with the results of the test throughout the six months prior to the blood sampling. An informed consent form was signed by the donor. This study was approved by the Guilan University of Medical Sciences Ethics Committee (Ethical code: IR.GUMS.REC.1399.338).

The MN assay in human lymphocytes was performed using whole blood cultures as previously described (14). Briefly, the blood sample was seeded in the 6-well culture plates with DMEM and PHA (to stimulate mitosis) supplemented with 10% (v/v) heat-inactivated FBS, 100 units/mL penicillin/streptomycin. Subsequently, the plates were incubated for 20 h under the conditions of 5% $CO_2$ air at 37 °C. The cultured blood samples were treated for 24 h as follows: (1) The control group treated with cosolvent and normal saline; (2 and 3) indomethacin groups treated only with indomethacin at 100 and 200 µM (15); (4 and 5) indomethacin-curcumin groups treated with indomethacin at 100 and 200 µM plus curcumin at 27 µM (16). Then, cytochalasin-B (3 µg/mL) was added to the samples, leading to the inhibition of cytokinesis. After 28 h, the culture was harvested, all groups were centrifuged (10 min at 146 *g*) and the supernatants were discarded. Then after, the samples were treated with hypotonic KCL (0.075 M) to lyse red blood cells. After fixing the samples with methanol/glacial acetic acid (3:1), the slides were prepared, stained with Giemsa solution (1.5%), and assessed randomly with an optical microscope (Micros Austria daffodil MCX100, Vienna, Austria). At least 1000 binucleated cells were counted at 400X magnification for each sample and micronuclei were determined.

*In vivo assessment of genotoxicity by MN assay in mice bone marrow cells*

Forty-two male Balb/c mice (3-4 weeks, 25-30 g body weight) were obtained from the Animal House of Guilan School of Pharmacy (Rasht, Iran). The animals were kept under controlled conditions, with free access to food and water, and in an environment with a 12/12-h light/dark cycle. All animal care and experiments were approved by the Ethics Committee of Guilan University of Medical Sciences (Ethical code: IR.GUMS.REC.1399.493).

MN method proceeded according to the previously described procedure (6). The mice fasted for 4 h before the experiment and were treated as follows (6 mice in each group): (1) Control group, mice received a single *i.p* injection of normal saline and cosolvent (tween 2%); (2-4) indomethacin treatment groups, mice received a single *i.p* dose of indomethacin at 10, 20, and 40 mg/kg (17); (5-7) indomethacin-curcumin treatment groups, mice received a single *i.p* dose of curcumin at 100 mg/kg (18) 30 min before a single *i.p* dose of indomethacin 10, 20, and 40 mg/kg.

Twenty-four hours after the treatment, the mice were euthanized with $CO_2$ asphyxiation according to ethical principles. Both femurs were removed, the muscles around them trimmed, and both ends of the femur were cut to collect the bone marrow cells. The bone marrow from both femurs was flushed in the form of a fine suspension into a centrifuge tube containing FBS and centrifuged at 200 *g* for 5 min. Then after, the supernatant was removed, and the cells were resuspended with the remained serum. The slides were prepared, stained with Giemsa solution (3%), and assessed randomly with an optical microscope (Micros Austria daffodil MCX100, Vienna, Austria). At least 2000 erythrocytes per mouse were counted at 1000X magnification and the ratio of PCE to NCE cells was determined.

***Oxidative stress evaluation***

*In vitro and in vivo assessment of lipid peroxidation*

In this study, the thiobarbituric acid method was used to determine the final products of lipid peroxidation (MDA) in human peripheral blood lymphocytes or mice bone marrow cells as reported earlier (19). Briefly, tris buffer was added to 1 mL of each homogenate sample (treated lymphocytes/mice bone marrow) and vortexed for 1 min. The samples were mixed with phosphoric acid and thiobarbituric acid reagent and placed in a Bain-Marie (100 °C, 30 min). Finally, the samples were placed on ice and 0.5 mL of n-butanol was added. After centrifugation of the samples, supernatant absorbance was determined at a wavelength of 535 nm with a microplate spectrophotometer reader (Epoch™ Microplate Spectrophotometer, BioTek, USA).

*In vitro and in vivo assessment of glutathione concentration*

Glutathione (GSH) concentration, as an intrinsic antioxidant, was assessed according to

Ellman's method in human peripheral blood lymphocytes or mice bone marrow cells as described previously (20). Thus, 1 mL of trichloroacetic acid solution (10%) was added to 1 mL of homogenate sample (treated lymphocytes/mice bone marrow) and homogenized using a vortex mixer. Then, it was centrifuged (for 15 min at 3500 rpm) and tris buffer and 5, 5′-dithiobis-(2-nitrobenzoic acid) (DTNB) solution was added to the mentioned solution. The final solution was homogenized using a vortex mixer and incubated in a dark place for 15 min. Finally, the samples were analyzed using a spectrophotometer at a wavelength of 412 nm (Perkin Elmer Company, Lambda 25 model, USA).

### *Data analysis*

The results are presented as the mean ± SD. Statistical analyses were performed using GraphPad Prism statistical software (version 6, USA). The normal distribution of all data was assessed using the Shapiro-Wilk test. As the distribution of all data was normal, statistical significance was determined using the one-way analysis of variance (ANOVA) test followed by the Tukey post hoc test. *P-values* < 0.05 were considered significant.

## RESULTS

### *In vitro MN assay in human peripheral blood lymphocytes*

Figure 1 shows a binucleated lymphocyte in the control group (Fig. 1A) and a binucleated lymphocyte with micronuclei in the indomethacin-treated group at 200 µM (Fig. 1B).

As shown in Fig. 2, the average micronuclei percentage observed in peripheral blood lymphocytes in indomethacin-treated groups (100 µM and 200 µM) significantly increased compared to the control group. The average percentage of micronuclei was significantly decreased in the group treated concomitantly with indomethacin (100 and 200 µM) and curcumin compared to the groups treated only with the same concentration of indomethacin.

### *In vivo MN assay in mice bone marrow cells*

As shown in Fig. 3, NCEs are mature erythrocytes stained in pink, and PCEs are observed as bluish tint immature form. Furthermore, micronuclei are DNA fragments separate from the main nucleus that are seen as dark blue spots in the PCE cytoplasm.

Table 1 shows that the average ratio of PCE to NCE in the indomethacin-treated groups at 20 and 40 mg/kg showed a significant decrease compared to the control group. Also, the average ratio of PCE/PCE + NCE to all erythrocytes in the indomethacin dosage of 40 mg/kg decreased significantly compared to the control group.

Furthermore, the average ratio of PCE to NCE in the group treated simultaneously with indomethacin (20 and 40 mg/kg) and curcumin (100 mg/kg) showed a significant increase compared to the group treated only with the same dose of indomethacin.

Figure 4 shows that the average micronuclei percentage observed in PCE cells in indomethacin-treated groups (20 and 40 mg/kg) increased significantly in comparison with the control group.

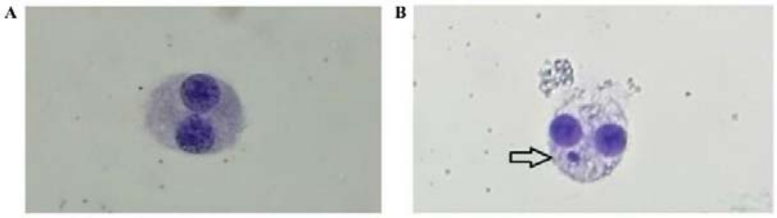


**Fig. 1.** Giemsa-stained binucleated human peripheral blood lymphocytes (magnification: 400×). (A) Control group (without any micronuclei); (B) the group treated with indomethacin at 200 µM (with micronuclei).

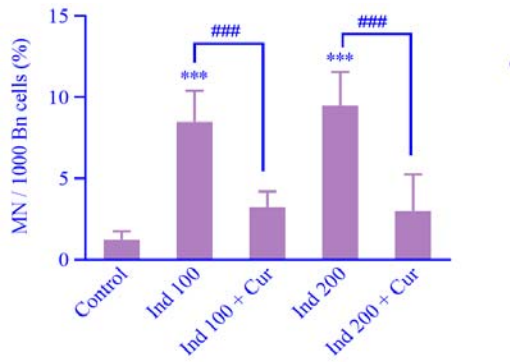


**Fig. 2.** The effect of indomethacin and curcumin (27 µg/mL) on average micronuclei percentage was observed in human peripheral blood lymphocytes. Data represent the mean ± SD of three separate experiments. ***$P < 0.001$ Indicates significant differences compared to the control group; $^{###}P < 0.001$ between the designated groups. MN, Micronucleus; Bn cells, binucleated cells; Ind, indomethacin; Cur, curcumin.

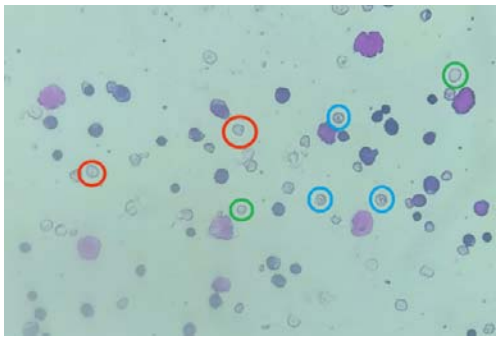

**Fig. 3.** Micronuclei induced in mice bone marrow cells exposed to indomethacin (magnification: 1000×). Green circles represent polychromatic erythrocytes; blue circles represent normochromatic erythrocytes; and red circles for monomicronucleated polychromatic erythrocytes.

**Table 1.** The effect of indomethacin and curcumin on the proportion of PCE/NCE and PCE/PCE+NCE observed in PCE cells. Data represent the mean ± SD of three separate experiments.

| Treatment | PCE/NCE | PCE/PCE+NCE |
|---|---|---|
| **Control** | 1.050 ± 0.065 | 0.511 ± 0.015 |
| **Indomethacin 10 mg/kg** | 0.866 ± 0.057* | 0.499 ± 0.068 |
| **Indomethacin 20 mg/kg** | 0.746 ± 0.050*** | 0.427 ± 0.016* |
| **Indomethacin 40 mg/kg** | 0.668 ± 0.045*** | 0.400 ± 0.016** |
| **Indomethacin 10 mg/kg + curcumin 100 mg/kg** | 0.997 ± 0.082 | 0.498 ± 0.020 |
| **Indomethacin 20 mg/kg + curcumin 100 mg/kg** | 0.947 ± 0.115$^{\$}$ | 0.485 ± 0.029 |
| **Indomethacin 40 mg/kg + curcumin 100 mg/kg** | 0.868 ± 0.068$^{\#}$ | 0.464 ± 0.019 |

*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ indicate significant differences compared to the control group; $^{\$}P < 0.05$ versus the group treated with indomethacin at 20 mg/kg; $^{\#}P < 0.05$ in contrast with the group treated with indomethacin at 40 mg/kg. PCE, Polychromatic erythrocyte; NCE, normochromic erythrocyte.

Table 1 shows that the average ratio of PCE to NCE in the indomethacin-treated groups at 20 and 40 mg/kg showed a significant decrease compared to the control group. Also, the average ratio of PCE/PCE + NCE to all erythrocytes in the indomethacin dosage of 40 mg/kg decreased significantly compared to the control group.

Furthermore, the average ratio of PCE to NCE in the group treated simultaneously with indomethacin (20 and 40 mg/kg) and curcumin (100 mg/kg) showed a significant increase compared to the group treated only with the same dose of indomethacin.

Figure 4 shows that the average micronuclei percentage observed in PCE cells in indomethacin-treated groups (20 and 40 mg/kg) increased significantly in comparison with the control group.

Also, the average micronuclei percentage observed in PCE cells in groups treated concomitantly with indomethacin (20 and 40 mg/kg) and curcumin (100 mg/kg) showed a significant decrease compared to the groups treated only with the same dose of indomethacin.

### *In vitro measurement of lipid peroxidation in human peripheral blood lymphocytes*

As shown in Fig. 5, the amount of MDA in blood lymphocytes in the indomethacin-treated groups (100 and 200 µM) increased significantly compared to the control group. This issue can indicate the induction of oxidative stress in high doses of indomethacin. Furthermore, the MDA level in blood lymphocytes in the group treated simultaneously with indomethacin (100 and 200 µM) and curcumin (27 µM) decreased significantly compared to the group treated with the same concentration of indomethacin.

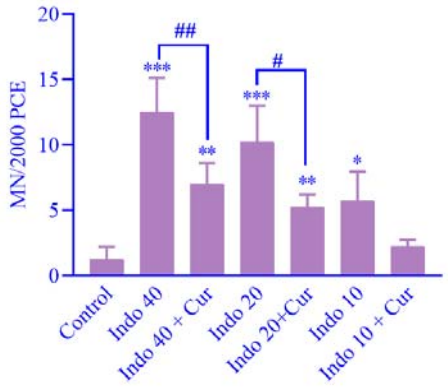


**Fig. 4.** The effect of indomethacin and curcumin (100 mg/kg) on average micronuclei percentage observed in PCE cells. Data represent the mean ± SD of three separate experiments. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001 indicate significant differences compared to the control group; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ between the designated groups. MN, Micronucleus; PCE, polychromatic erythrocyte; Indo, indomethacin; Cur, curcumin.

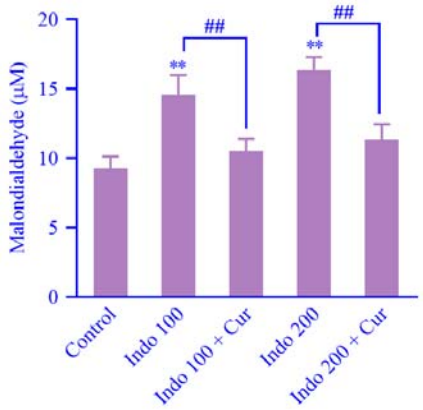


**Fig. 5.** The effect of indomethacin and curcumin (27 µg/mL) on lipid peroxidation was observed in human peripheral blood lymphocytes. Data represent the mean ± SD of three separate experiments. ***P* < 0.01 Indicates significant differences compared to the control group; $^{\#\#}P < 0.01$ between the designated groups. Indo, Indomethacin; Cur, curcumin.

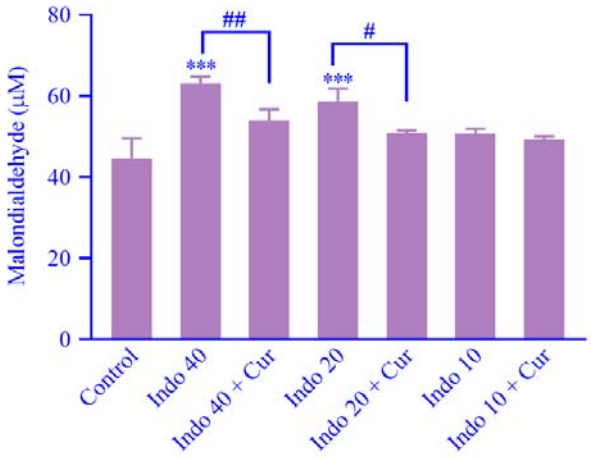


**Fig. 6.** The effect of indomethacin and curcumin (100 mg/kg) on lipid peroxidation was observed in mice bone marrow cells. Data represent the mean ± SD of three separate experiments. ****P* < 0.001 Indicates significant differences compared to the control group; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ between the designated groups. Indo, Indomethacin; Cur, curcumin.

### *In vivo measurement of lipid peroxidation in mice bone marrow cells*

Figure 6 shows that the level of MDA in bone marrow cells in the indomethacin-treated groups (20 and 40 mg/kg) increased significantly compared to the control group. This issue can indicate indomethacin-induced oxidative stress.

The level of MDA in bone marrow cells in the groups treated concomitantly with indomethacin (20 and 40 mg/kg) and curcumin (100 mg/kg) decreased significantly in comparison with the groups treated only with the same dose of indomethacin.

***In vitro measurement of GSH concentration in human peripheral blood lymphocytes***

As shown in Fig. 7, the level of GSH in blood lymphocytes in the indomethacin (100 µM)-treated group reduced significantly compared to the control group. This reduction was much more significant in the group treated with indomethacin at 200 µM. The obtained results can indicate the induction of oxidative stress in high concentrations of indomethacin.

In addition, the amount of GSH in blood lymphocytes treated concomitantly with indomethacin (200 µM) and curcumin (27 µM) significantly increased () compared to the groups treated only with the same concentration of indomethacin.

***In vivo measurement of GSH concentration in mice bone marrow cells***

Figure 8 shows the amount of GSH in bone marrow cells of indomethacin (10, 20, and 40 mg/kg) -treated groups was significantly reduced in comparison with the control group. The obtained results can indicate the indomethacin-induced oxidative stress.

Furthermore, the level of GSH in bone marrow cells of the group treated simultaneously with indomethacin at 40 mg/kg and curcumin at 100 mg/kg significantly increased in comparison with the group treated only with indomethacin at 40 mg/kg.

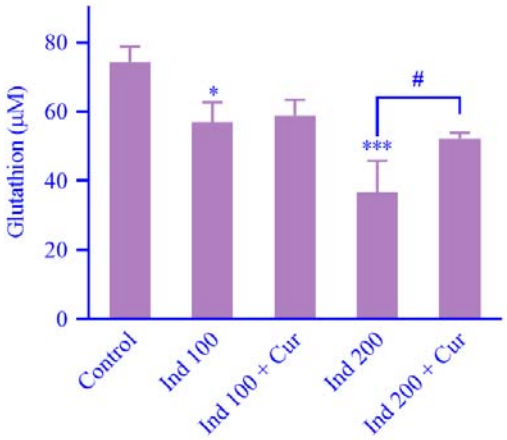


**Fig. 7.** The effect of indomethacin and curcumin (27 µg/mL) on glutathione concentration was observed in human peripheral blood lymphocytes. Data represent the mean ± SD of three separate experiments. **P* < 0.05 and ****P* < 0.001 indicate significant differences compared to the control group; #*P* < 0.05 between the designated groups. Indo, Indomethacin; Cur, curcumin.

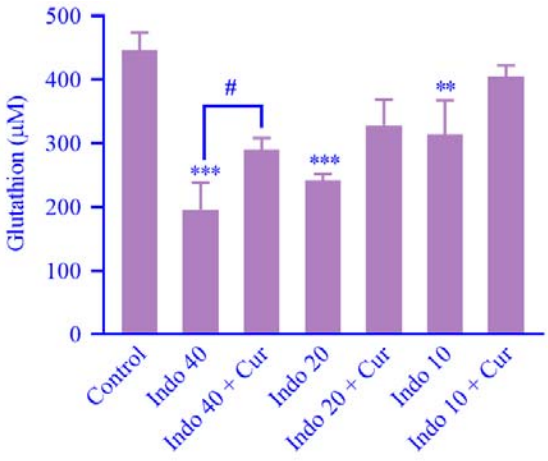


**Fig. 8.** The effect of indomethacin and curcumin (100 mg/kg) on glutathione concentration was observed in mice bone marrow cells. Data represent the mean ± SD of three separate experiments. ***P* < 0.01 and ****P* < 0.001 indicate significant differences compared to the control group; #*P* < 0.05 between the designated groups. Indo, Indomethacin; Cur, curcumin.

## DISCUSSION

In the present study, an MN assay *in vitro* (human peripheral blood lymphocytes) and *in vivo* (mice bone marrow cells) has been conducted to explore the genotoxicity of indomethacin. The obtained results showed the average micronuclei percentage in indomethacin-treated groups increased in comparison with the control group, which may indicate the indomethacin-induced genotoxicity. The data obtained in our study are in agreement with the results of another study, which showed the genotoxic effects of indomethacin-loaded nanocapsules on the lymphocyte and HepG2 cell lines (21). The same study showed that indomethacin at a concentration of 500 µg/mL caused an increase in micronuclei number and also led to a small but statistically significant clastogenic effect (breakage in the chromosome) in HepG2 cells. Moreover, in another study conducted by Ferreira *et al.* the indomethacin-induced genotoxicity in tumorized male C57BL/6 mice was investigated *via* micronucleus assay in the bone marrow cells, and the results showed that indomethacin was genotoxic at the dose higher than 2.5 mg/kg after 5 consecutive days treatment (17).

The excess ROS are also known to be a genotoxic stress that can induce DNA damage and mutation following ineffective repair of DNA damage (5). Therefore, to have a better understanding of the possible genotoxic mechanism of indomethacin, we also evaluated the MDA and GSH levels, as key oxidative stress indicators (22), in both *in vitro* and *in vivo*. Our findings revealed that indomethacin increases the level of MDA and decreases the level of GSH compared to the control group. These data support the idea that one of the possible mechanisms of indomethacin-induced genotoxicity might be through increasing the level of oxidative stress. In agreement with our data, Ahmad *et al*. found a significant increase in the number of micronuclei in PCE cells in the groups treated with indomethacin (23). Notably, the same study also reported a significant decrease in antioxidant enzymes and a remarkable increase in lipid peroxidation. Therefore, oxidative stress caused by indomethacin has been mentioned as one of the possible reasons for genotoxicity. Moreover, Maity *et al.* observed that the indomethacin treatment in an indomethacin-induced gastric damage model leads to the development of mitochondrial oxidative stress as indicated by increased depletion of mitochondrial GSH content and augmentation of mitochondrial lipid peroxidation, indicated by increased MDA (24). In another study on gastric ulcers caused by chronic use of indomethacin, similar results were obtained (25). It was reported that the main reason for the observed effect was the increment of MDA level and free radicals including ROS (25).

In this work, we also examined the protective effect of curcumin, as a safe plant-derived antioxidant, against genotoxicity and oxidative stress induced by indomethacin. Results of the MN assay revealed that the simultaneous use of curcumin and indomethacin in both *in vitro* (human peripheral blood lymphocytes) and *in vivo* (mice bone marrow cells) models reduced the number of micronuclei compared to the group treated only with indomethacin. These data might indicate the potential protective effect of curcumin against genotoxicity caused by indomethacin. In addition, the assessment of oxidative stress markers found that the concomitant use of curcumin and indomethacin in both *in vitro* and *in vivo* models decreases the level of MDA and increases the level of GSH compared to the group treated only with indomethacin. These results support the idea that one of the possible mechanisms of curcumin for protection against indomethacin-induced genotoxicity might be through reducing the level of oxidative stress. The results of our study are consistent with the study conducted by Corona-Rivera *et al*. investigated the protective effects of curcumin against copper-induced genotoxicity. The results of their study, which was done *via* MN and comet assay, revealed that curcumin has genoprotective effects against DNA damage induced by high copper concentration (26). Sankar *et al*. using MN and comet assay showed that curcumin at a dose of 100 mg/kg reduces significantly micronuclei formation and DNA damage caused by cypermethrin (18).

In line with the above-mentioned studies, Eke *et al.* investigated the protective role of curcumin against DNA damage caused by perfluorooctane sulfonate injection in rat liver cells. They reported that curcumin at a dose of 80 mg/kg had a protective role against DNA damage and also reduced the number of micronuclei (27).

## CONCLUSION

Taken together, this study showed that curcumin might have a potential protective effect against the genotoxicity of indomethacin in both *in vitro* (human peripheral blood lymphocytes) and *in vivo* (mice bone marrow cells) models. Part of this effect might be through modulating oxidative stress markers, such as a reduction in MDA level and an increment in the level of GSH. Therefore, the antioxidant properties of curcumin make it an attractive agent to inhibit or treat pathological conditions related to indomethacin-induced genotoxicity. However, the use of other genotoxicity testing techniques such as the Comet assay, measuring the amount of other oxidative stress factors such as ROS can help a better understanding of the molecular mechanisms involved. Moreover, histopathological assessment of bone marrow cells and other tissues subjected to the genotoxic damage of indomethacin is required to determine the appropriate curcumin dosage regimen as a potential genoprotective candidate.

### *Acknowledgments*

The data provided in this research was part of two Pharm.D. theses and financially supported by the Research Council of Guilan University of Medical Sciences, Rasht, Iran (Registration code: IR.GUMS.REC.1399.338 and IR.GUMS.REC.1399.493).

### *Conflict of interest statement*

The authors declared no conflicts of interest in this study.

### *Authors' contributions*

M. Evazalipour and E. Zamani contributed to the conceptualization, study design, project supervision, and manuscript writing. E. Zamani contributed to the data analysis and interpretation. R. Alipour Klour and A. Gholami Shekarsaraye contributed to the experimental studies and data acquisition. Foad Ghazizadeh contributed to the study design, experimental studies, and manuscript writing. The finalized article was read and approved by all authors.

## REFERENCES

1. Nalamachu S, Wortmann R. Role of indomethacin in acute pain and inflammation management: a review of the literature. Postgrad Med. 2014;126(4):92-97. DOI: 10.3810/pgm.2014.07.2787.
2. Lucas S. The pharmacology of indomethacin. Headache. 2016;56(2):436-446. DOI: 10.1111/head.12769.
3. Basivireddy J, Jacob M, Balasubramanian K. Indomethacin induces free radical-mediated changes in renal brush border membranes. Arch Toxicol. 2005;79(8):441-450. DOI: 10.1007/s00204-005-0658-4.
4. Waris G, Ahsan H. Reactive oxygen species: role in the development of cancer and various chronic conditions. J Carcinog. 2006;5:14,1-8. DOI: 10.1186/1477-3163-5-14.
5. Sedelnikova OA, Redon CE, Dickey JS, Nakamura AJ, Georgakilas AG, Bonner WM. Role of oxidatively induced DNA lesions in human pathogenesis. Mutat Res. 2010;704(1-3):152-159. DOI: 10.1016/j.mrrev.2009.12.005.
6. Kasamoto S, Masumori SH, Hayashi M. *In vivo* micronucleus assay in mouse bone marrow and peripheral blood. In: Dhawan A, Bajpayee M, editors. Genotoxicity assessment: methods and protocols. 1st ed. New York: Humana Totowa; 2013. pp: 179-190.
7. Minaiyan M, Sadraei H, Yousefi I, Sajjadi SE, Talebi A. Evaluation of the effect of hydroalcoholic and flavonoid-enriched extracts of *Dracocephalum kotschyi* on indomethacin-induced gastric ulcer in rats. Res Pharm Sci. 2021;16(2):141-152. DOI: 10.4103/1735-5362.310521.
8. Chung YJ, Robert C, Gough SM, Rassool FV, Aplan PD. Oxidative stress leads to increased mutation frequency in a murine model of myelodysplastic syndrome. Leuk Res. 2014;38(1):95-102. DOI: 10.1016/j.leukres.2013.07.008.
9. Chakraborty S, Kar SK, Roy K, Sengupta C. Exploring effects of different nonsteroidal antiinflammatory drugs on malondialdehyde profile. Acta Pol Pharm. 2006;63(2):83-88. PMID: 17514869.
10. Aggarwal BB, Harikumar KB. Potential therapeutic effects of curcumin, the anti-inflammatory agent, against neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases. Int J Biochem Cell Biol. 2009;41(1):40-59. DOI: 10.1016/j.biocel.2008.06.010

11. Sökmen M, Akram Khan M. The antioxidant activity of some curcuminoids and chalcones. Inflammopharmacology. 2016;24(2):81-86. DOI: 10.1007/s10787-016-0264-5.
12. Sahebkar A, Serban M-C, Ursoniu S, Banach M. Effect of curcuminoids on oxidative stress: a systematic review and meta-analysis of randomized controlled trials. J Funct Foods. 2015;18:898-909. DOI: 10.1016/j.jff.2015.01.005.
13. Sudjarwo SA, Sudjarwo GW. Protective effect of curcumin on lead acetate-induced testicular toxicity in Wistar rats. Res Pharm Sci. 2017;12(5):381-390. DOI: 10.4103/1735-5362.213983.
14. Fenech M. Cytokinesis-block micronucleus cytome assay. Nat Protoc. 2007;2(5):1084-1104. DOI: 10.1038/nprot.2007.77.
15. Kang Y, Wu J, Yin G, Huang Z, Yao Y, Liao X, *et al*. Preparation, characterization and *in vitro* cytotoxicity of indomethacin-loaded PLLA/PLGA microparticles using supercritical $CO_2$ technique. Eur J Pharm Biopharm. 2008;70(1):85-97. DOI: 10.1016/j.ejpb.2008.03.011.
16. Shafaghati N, Hedayati M, Hosseinimehr SJ. Protective effects of curcumin against genotoxicity induced by 131-iodine in human cultured lymphocyte cells. Pharmacogn Mag. 2014;10(38):106-110. DOI: 10.4103/0973-1296.131020.
17. Ferreira NH, Ribeiro AB, Rinaldi-Neto F, Fernandes FS, do Nascimento S, Braz WR, *et al*. Anti-melanoma activity of indomethacin incorporated into mesoporous silica nanoparticles. Pharm Res. 2020;37(9):172,1-11. DOI: 10.1007/s11095-020-02903-y.
18. Sankar P, Telang AG, Manimaran A. Curcumin protects against cypermethrin-induced genotoxicity in rats. Environ Toxicol Pharmacol. 2010;30(3):289-291. DOI: 10.1016/j.etap.2010.07.005.
19. Zamani E, Mohammadbagheri M, Fallah M, Shaki F. Atorvastatin attenuates ethanol-induced hepatotoxicity *via* antioxidant and anti-inflammatory mechanisms. Res Pharm Sci. 2017;12(4):315-321. DOI: 10.4103/1735-5362.212049.
20. Arab-Nozari M, Zamani E, Latifi A, Shaki F. Mitochondrial toxicity of aluminium nanoparticles in comparison to its ionic form on isolated rat brain mitochondria. Bratisl Lek Listy. 2019;120(7):516-522. DOI: 10.4149/BLL_2019_083.
21. Froder J, Dupeyrón D, Carvalho J, Maistro E. *In vitro* study of the cytotoxic and genotoxic effects of indomethacin-loaded Eudragit® L 100 nanocapsules. Genet Mol Res. 2016;15(3):1-12. DOI: 10.4238/gmr.15038727.
22. Katerji M, Filippova M, Duerksen-Hughes P. Approaches and methods to measure oxidative stress in clinical samples: research applications in the cancer field. Oxid Med Cell Longev. 2019;2019:1279250,1-30. DOI: 10.1155/2019/1279250.
23. Ahmad MH, Fatima M, Hossain MM, Mondal AC. Determination of potential oxidative damage, hepatotoxicity, and cytogenotoxicity in male Wistar rats: role of indomethacin. J Biochem Mol Toxicol. 2018;32(12):e22226,1-8. DOI: 10.1002/jbt.22226.
24. Maity P, Bindu S, Dey S, Goyal M, Alam A, Pal C, *et al*. Indomethacin, a non-steroidal anti-inflammatory drug, develops gastropathy by inducing reactive oxygen species-mediated mitochondrial pathology and associated apoptosis in gastric mucosa: a novel role of mitochondrial aconitase oxidation. J Biol Chem. 2009;284(5):3058-3068. DOI: 10.1074/jbc.M805329200.
25. Mesmine KTM, George EOE, Christophe M, Ernestine NZ, Paul TV, Barthelemy N. Aqueous extract of *Enantia chlorantha* (Annonaceae) prevents the delay in chronic gastric ulcer healing caused by indomethacin in rats. Br J Pharm Res. 2015;8:1-13. DOI: 10.9734/BJPR/2015/19035.
26. Corona-Rivera A, Urbina-Cano P, Bobadilla-Morales L, Vargas-Lares JdJ, Ramírez-Herrera MA, Mendoza-Magaña ML, *et al*. Protective *in vivo* effect of curcumin on copper genotoxicity evaluated by comet and micronucleus assays. J Appl Genet. 2007;48(4):389-396. DOI: 10.1007/BF03195238.
27. Eke D, Celik A, Yilmaz MB, Aras N, Sel SK, Alptekin D. Apoptotic gene expression profiles and DNA damage levels in rat liver treated with perfluorooctane sulfonate and protective role of curcumin. Int J Biol Macromol. 2017;104:515-520. DOI: 10.1016/j.ijbiomac.2017.06.075.