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## ORIGINAL PAPER

## *Corchorus olitorius* (jute) extract induced cytotoxicity and genotoxicity on human multiple myeloma cells (ARH-77)

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### Abstract

**Context:** *Corchorus olitorius* L. (Malvaceae) has industrial importance in world jute production and is a widely cultivated and consumed crop in Cyprus and in some Arabic countries.

**Objective:** The present study investigated cytotoxic and genotoxic effects of leaf extracts (LE) and seed extracts (SE) of the *C. olitorius* on the multiple myeloma-derived ARH-77 cells. The extracts were also evaluated for their total phenol content (TPC) and free radical scavenging activity (FRSA).

**Materials and methods:** *C. olitorius* was collected from Nicosia, Cyprus. TPC and FRSA were measured by Folin-Ciocalteu and DPPH free radical methods, respectively. Cytotoxicity was evaluated by the MTT assay (4–2048 µg/mL range), and DNA damage (at IC<sub>50</sub> and ½IC<sub>50</sub>) was measured by the comet assay.

**Results and discussion:** The LE had significantly higher total phenol (78 mg GAE/g extract) than the SE (2 mg GAE/g extract) with significantly higher FRSA (IC<sub>50</sub> LE: 23 µg/mL and IC<sub>50</sub> SE: 10 401 µg/mL). Both LE and SE exerted cytotoxic effects on cells after 48 h. The IC<sub>50</sub> of SE (17 µg/mL) was lower than LE (151 µg/mL), which demonstrates its higher cytotoxicity on cells. The extracts were applied at 150 and 75 µg/mL for LE and at 17 and 8.5 µg/mL for SE, and the results of the comet assay revealed that the extracts induced genotoxic damage on ARH-77 cells. In both 48 h leaf and seed extract treatments, genotoxic damage significantly increased with increasing concentrations at relevant cytotoxic concentrations.

**Conclusion:** To our knowledge, this is the first report demonstrating the high cytotoxic potential of *C. olitorius* SE and the genotoxic potential of LE and SE.

### Keywords

Antiproliferative activity, DNA damage, leaf, seed

### History

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### Introduction

*Corchorus* (Malvaceae), a genus of about 40–100 species of flowering plants, is distributed in the tropics of both hemispheres. In North Cyprus, this genus is represented with two annual species, namely *Corchorus olitorius* L. and *C. trilobularis* L. *C. olitorius* (Jute) is a native plant of tropical Africa and Asia and has since spread to Australia, South America, and some parts of Europe (Meikle, 1977). Besides having industrial importance in world jute production, it has agricultural importance as a widely cultivated and consumed crop in Cyprus, and some Arabic countries under the Arabic name “Molukhyia”. It has traditional uses for the treatment of fever, chronic cystitis, aches and pains, dysentery, enteritis, and pectoral pains (Zakaria et al., 2006).

Plant-derived compounds comprise diverse biological activities with different mechanisms of actions. Some studied biological activities of different parts of *Corchorus olitorius* L.

are cardiovascular, antihistaminic, hepatobiliary, renal, anti-convulsant, antiestrogenic, antimalarial, and hematological changes (Khan et al., 2006). Nishiumi et al. (2006) demonstrated suppressive effect of ethanol extract on aryl hydrocarbon receptor (AhR) transformation induced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. In addition, both hydrophilic and lipophilic extracts of the leaf have shown antioxidant activity (Oboh et al., 2009).

Medically screened plants that are used as traditional remedies increase the chance of finding new bioactive principles. Epidemiological studies have suggested that the medicinal plants can have considerable anticarcinogenic effect, and as well inhibiting the genotoxicity and carcinogenicity of anticancer drug to normal cells (Newman et al., 2003). It is important to screen anticarcinogenic potential of plants, either in the form of crude extracts or as components isolated from them. Screening of anticancer activities of various plants comprises the basis of studies on the pharmacological mechanisms and searching for chemical structures from herbal extract for new anticancer drugs (Cragg & Newman, 1999). A number of other plant-derived compounds such as vinblastine, vincristine, etoposide, and taxotere are currently used as anticancer drugs.

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The present study investigated *in vitro* effects of LE and SE of *C. olitorius* on the growth of multiple myeloma-derived ARH-77 cells with their genotoxic potential. The extracts were also evaluated for their total phenol content (TPC) and free radical scavenging activity (FRSA).

## Materials and methods

### Plant material and extraction

Leaves and seeds of *C. olitorius* were collected from Nicosia, Cyprus, in August and October (2010), respectively. Voucher specimens were identified by Dr. Evren Cabi (Department of Biology, Namik Kemal University, Tekirdağ, Turkey) and stored as herbarium materials (NGBB 3935) at the Nezahat Gökyiğt Botanical Garden, İstanbul, Turkey. Plants were also cultured from seeds in the Greenhouse of Institute of Transplantation and Gene Sciences, Baskent University (Kazan-Ankara, Turkey). Dry material was powdered by using a coffee blender. Powder (10 g) was mixed with 100 mL of pure methanol (Merck, Darmstadt, Germany) and incubated for 24 h at room temperature (dark) with continuous shaking. The solution was filtered (Whatman No. 40), and the filtrate was lyophilized by using a freeze-dryer at  $-50^{\circ}\text{C}$ , 0.50 hPa (LyoPro 3000; Thermo Scientific (Heto), Waltham, MA). Dry material was recovered in dimethyl sulfoxide (DMSO; Sigma-Aldrich, St. Louis, MO) and stored at  $-20^{\circ}\text{C}$ . Extract yields [(g lyophilized material/10 g powder)  $\times$  100] were calculated as 15% and 21% for LE and SE, respectively.

### Analysis of TPC of the extracts

The Folin–Ciocalteu method was used to assay total phenol (Folin & Ciocalteu, 1927; Slinkard & Singleton, 1977). Two microliters of sample (0.05 g/mL), 50  $\mu\text{L}$  Folin's reagent (Sigma-Aldrich), and 300  $\mu\text{L}$  10% (w/v) sodium carbonate (Sigma-Aldrich) were sequentially added to 1 mL assay mixture, and the mixture was incubated at  $40^{\circ}\text{C}$  in a water bath for 30 min. The absorbance was measured at 765 nm, and the TPC was represented as mg gallic acid (Sigma-Aldrich) equivalents (GAE) per g extract using gallic acid calibration curve. The assays were performed as triplicate experiments.

### DPPH radical scavenging assay

FRSA of the plant extracts have been performed using DPPH radical. The method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to the formation of the nonradical form DPPH-H that is measured spectrophotometrically according to decoloration of purple-colored solution of DPPH (Sharma & Bhat, 2009). Three milliliters of serial extract dilutions were mixed with 1 mL 200  $\mu\text{M}$  methanol solution of DPPH $^{\bullet}$  (Sigma-Aldrich), vortexed, and incubated at room temperature (dark) for 30 min. The absorbance was measured at 517 nm. Inhibition of DPPH $^{\bullet}$  was calculated as:

$$I\% = (A_{\text{blank}} - A_{\text{sample}}/A_{\text{blank}}) \times 100.$$

Inhibitory concentration 50 (IC<sub>50</sub>) of extracts were calculated from trend lines of I versus extract concentration plots. Butylated hydroxytoluene (BHT; Sigma-Aldrich),

L-ascorbic acid (AscA; Sigma-Aldrich), and  $\alpha$ -tocopherol ( $\alpha$ -Toc; Sigma-Aldrich) were used as positive controls in the assays. The assays were performed as triplicate experiments.

### Cell culture

Multiple myeloma-derived ARH-77 cells were maintained in RPMI 1640 medium (Biochrom AG, Berlin, Germany) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Biochrom AG), 2 mM L-glutamine (Biochrom AG), and streptomycin (100  $\mu\text{g}/\text{mL}$ )–penicillin (100 U/mL) mix (Biological Industries, Kibbutz Beit-Haemek, Israel) at  $37^{\circ}\text{C}$  in a humidified atmosphere of 5% CO<sub>2</sub> (Heraeus, Hanau, Germany).

### Assay for cytotoxicity

The effects of LE and SE on the proliferation of ARH-77 cells were colorimetrically tested by biochemical reduction of MTT [3-(4,5-dimethyl-2-thiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide; Sigma-Aldrich]. The extracts were diluted from 2048  $\mu\text{g}/\text{mL}$  to 4  $\mu\text{g}/\text{mL}$  horizontally in 96-well microtiter plates. DMSO cytotoxicity was tested at the solvent dilution range. Ten thousand cells were seeded to each well with the exception of medium control wells. The plates were incubated for 48 h, and then 20  $\mu\text{L}$  of MTT solution (5 mg/mL) were added to each well. After incubation for 4 h, 100  $\mu\text{L}$  sodium dodecyl sulfate (Sigma-Aldrich) solution (10% w/v) were added to each well. The plates were further incubated overnight to allow the dissolution of formazan crystals that were produced by the mitochondrial activity of viable cells. The inhibition of cell proliferation was determined by measuring the optical density of the chromogenic product at 540 nm with an ELISA reader (Biotek Instrument ELx800; Winooski, VT). Inhibition of cell proliferation and inhibitory concentration 50 (IC<sub>50</sub>) values, which are concentrations at which 50% of cells are viable, were calculated from the logarithmic trend lines of the viability graphs.

### Alkaline comet assay (alkaline single cell gel electrophoresis)

One of the quantitative parameters to measure DNA damage induced by various agents in cells is the comet assay (Yurtcu et al., 2011, 2012). Extracts were applied to ARH-77 cells at IC<sub>50</sub> and half concentrations (IC<sub>50</sub>) as 150 and 75  $\mu\text{g}/\text{mL}$  for LE, and 17 and 8.5  $\mu\text{g}/\text{mL}$  for SE, respectively, for 48 h. Control groups were assayed without any treatment in each experiment. The highest DMSO concentration was also applied in separate flasks (DMSO controls), in order to determine solvent effect on experimental setups. For the determination of genotoxic effects of treatments on ARH-77 cells, alkaline single cell gel electrophoresis (SCGE) was performed as previously described (O'Brien et al., 2000). In brief, cells were resuspended in 0.5 mL phosphate buffered saline (PBS; Sigma-Aldrich), and 5  $\mu\text{L}$  of cell suspension was mixed with 35  $\mu\text{L}$  of 1% (w/v) low-melting point agarose (LMPA; Sigma-Aldrich) and added on to the slides coated with 0.5% (w/v) normal melting point agarose (NMPA; Sigma-Aldrich). Coverslips were placed and slides were incubated on ice packs until the solidification of the agarose.

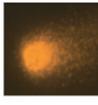
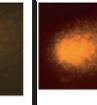
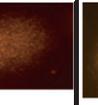
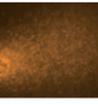
Comet score	0	1+	2+	3+	4+
400X					
AU	0xcell# <sub>0</sub>	1xcell# <sub>1</sub>	2xcell# <sub>2</sub>	3xcell# <sub>3</sub>	4xcell# <sub>4</sub>

Figure 1. Evaluation of comet results ( $\times 400$ ). Nuclei were scored as 0, 1+, 2+, 3+ and 4+ according to apparent relative proportion of DNA in the tail and head. Arbitrary units (AU) for quantification were obtained by multiplying counted nuclei by their score.

Coverslips were removed, and 40  $\mu\text{L}$  1% (w/v) LMPA were added on to the slides. Slides were incubated in lysis solution (2.5 M NaCl, 100 mM ethylenediaminetetraacetic acid (EDTA) disodium salt, 10 mM Tris; pH 10) at 4 °C (dark) for 2 h. Slides were incubated in electrophoresis buffer (300 mM NaOH, 1 mM (EDTA) disodium salt; pH > 13) for 20 min at dark, and electrophoresis was performed at 24 V (300 mA) for 30 min. After neutralization (0.4 M Tris; pH 7.5), slides were stained with 2  $\mu\text{g}/\text{mL}$  ethidium bromide and observed under a fluorescence microscope (Eclipse 600, Nikon, Tokyo, Japan) with 400 $\times$  magnification. A minimum of three SCGE slides were prepared for each treatment, and 100 nuclei were blindly scored per slide. Tail moment of DNA obtained by comet assay were expressed as arbitrary units (AU) in the present study, which were evaluated by visual scoring of nuclei representing DNA damage (Figure 1) (Zhao et al., 2006). Nuclei were scored as 0, 1+, 2+, 3+, and 4+ by a blinded observer according to apparent relative proportion of DNA in the tail and head (Figure 1). Each counted nucleus was multiplied by its score, and total scores were expressed as AU.

### Statistical analysis

All data are expressed as mean  $\pm$  standard error of the means (SEM). All statistical analyses were performed using SPSS Software (SPSS Inc., Chicago, IL). Treatments were statistically evaluated by one-way analysis of variance at 0.05 levels, and post-hoc Tukey analysis was carried out to find groups whose mean differences were significant. The results were subjected to two-tailed *t*-test where necessary ( $\alpha = 0.05$ ).

## Results

### TPC of the extracts

The TPC of the extracts is given in Table 1 in terms of mg GAE per g extract. The LE of *C. olitorius* had significantly higher level of TPC than the SE ( $p < 0.05$ ).

### Free radical scavenging activity

The LE ( $\text{IC}_{50}$ : 22.7  $\mu\text{g}/\text{mL}$ ) had significantly higher FRSA than SE ( $p < 0.05$ ) (Table 1).  $\text{IC}_{50}$  (FRSA) of standards AscA, BHT, and  $\alpha$ -Toc were 5.9  $\pm$  0.3, 12.6  $\pm$  0.5, and 5.3  $\pm$  0.5  $\mu\text{g}/\text{mL}$ , respectively.

Table 1. TPC,  $\text{IC}_{50}$  of DPPH\* scavenging activity, and  $\text{IC}_{50}$  of cytotoxicity.

Extracts	TPC (mg GAE*/g extract)	$\text{IC}_{50}$ (FRSA) ( $\mu\text{g}/\text{mL}$ )	$\text{IC}_{50}$ (cytotoxicity) ( $\mu\text{g}/\text{mL}$ )
Leaf	78.1 $\pm$ 1.0	22.7 $\pm$ 1.8	150.6 $\pm$ 0.9
Seed	2.2 $\pm$ 0.5	1040.8 $\pm$ 11.0	17.0 $\pm$ 1.1

GAE, gallic acid equivalents.

All values significantly differ between leaf and seed extracts ( $p < 0.05$ ).

### Cytotoxic effects of extracts on ARH-77 Cells

Both LE and SE exerted cytotoxic effects on ARH-77 cells in a range of 4–2048  $\mu\text{g}/\text{mL}$  (Figure 2). The solvent DMSO did not have any cytotoxic effect in the dilution range. The  $\text{IC}_{50}$  of SE (17  $\mu\text{g}/\text{mL}$ ) was  $\sim$ 9-fold lower than LE (Table 1), which demonstrates its higher cytotoxicity on ARH-77 cells.

### LE and SE caused dose-dependent genotoxic damage on ARH-77 cells

The visual analysis of comet assay measures the amount of DNA in the head and in the tail based on the principle that migration of nuclei with intact and damaged DNA on agarose differs (Figure 1). The results obtained with the comet assay are summarized in Figure 3. Both the extracts were applied at their respected  $\text{IC}_{50}$  and half of  $\text{IC}_{50}$  and induced genotoxic damage on ARH-77 cells. The control groups had 8  $\pm$  0.4, whereas it increased to 81.3  $\pm$  3.7 and 136.5  $\pm$  3.5 after 48 h of 1/2  $\text{IC}_{50}$  (75  $\mu\text{g}/\text{mL}$ ) and  $\text{IC}_{50}$  (150  $\mu\text{g}/\text{mL}$ ) leaf extract treatments, respectively. In concordance, genotoxic damage increased to 77.0  $\pm$  1.0 and 133.7  $\pm$  3.5 after 48 h of 1/2  $\text{IC}_{50}$  (8.5  $\mu\text{g}/\text{mL}$ ) and  $\text{IC}_{50}$  (17  $\mu\text{g}/\text{mL}$ ) seed extract treatments, respectively. In both leaf and seed extract treatments, genotoxic damage significantly ( $p < 0.05$ ) increased with increasing concentrations at relevant cytotoxic concentrations. DMSO used as solvent control did not cause significant increase in DNA damage.

## Discussion

There is a good positive correlation between antioxidant capacity and TPC of spices, medicinal herbs, and other

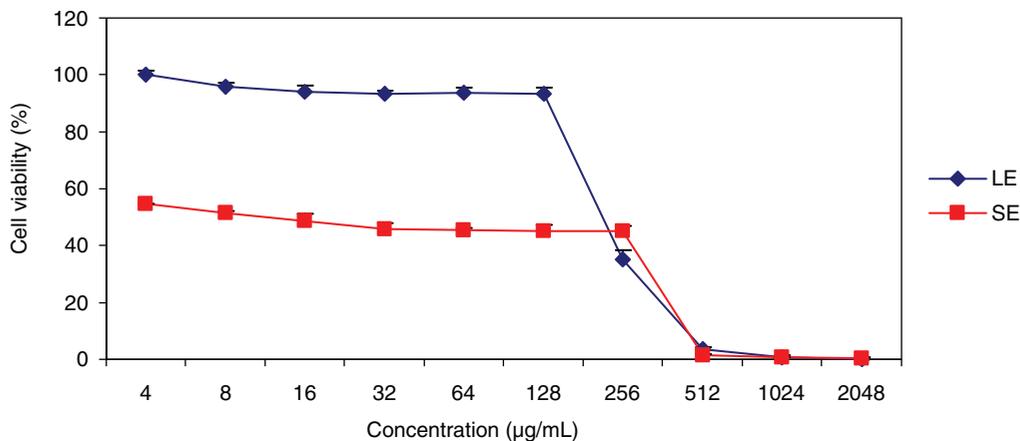


Figure 2. Effect of LE and SE on ARH-77 cell viability. SEM were obtained from three replicate plates.

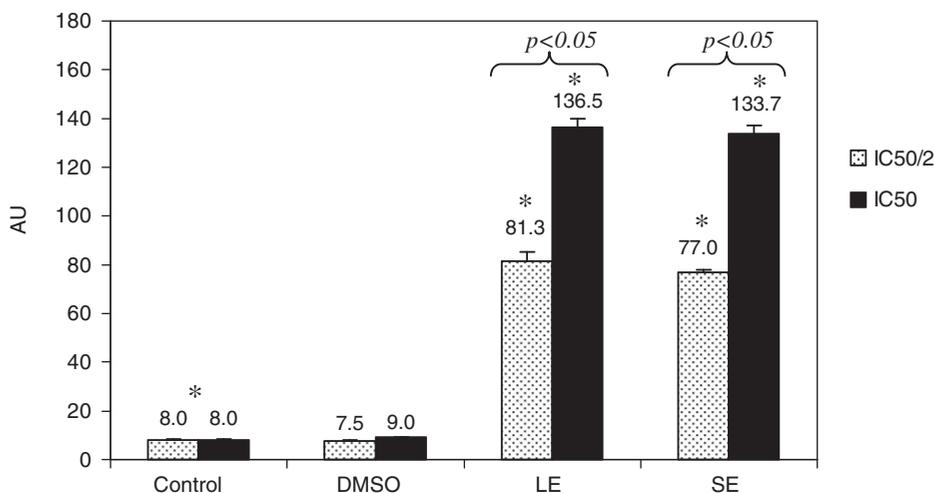


Figure 3. Genotoxic damage induced by LE and SE. \*Significant differences between control and other treatment groups.  $p$  Values represent significant difference between  $1/2$   $IC_{50}$  and  $IC_{50}$  LE and SE treatment groups.

dietary plants due to their electron transfer/hydrogen donating ability (Lu et al., 2011). In our study, phenol content of the extracts was well correlated to DPPH<sup>•</sup> scavenging activity. Phenol contents and FRSA of LE were considerably higher than the SE. Moreover, FRSA of the LE (i.e.,  $IC_{50}$  of  $22.7 \pm 1.8$  µg/mL) was comparable to the activity of natural and synthetic antioxidants tested. Azuma et al. (1999) identified 5-caffeoylquinic acid to be the most predominant antioxidant of *C. olitorius* leaves. Furthermore, flavonoid glycosides (astragalin, isoquercitrin, tolfolin and jugulanin), and coumarin glycosides (4,7-dihydroxycoumarin, cichoiriine and scopolin) besides quercetin derivatives have been identified in *C. olitorius* leaves and seeds (Mukherjee et al., 1998; Khan et al., 2006).

Concentration and time-dependent pro-oxidant effects of many antioxidants on cancer cells have been demonstrated (Bjelakovic et al., 2004; Woods et al., 1999; Zhang & Omaye, 2001). According to Table 1, LE with considerably higher TPC and FRSA had lower cytotoxic effects on cells, at concentration which was above the  $IC_{50}$  of FRSA. Higher concentrations of LE may be required to shift to pro-oxidant effects and exert cytotoxicity. On the other hand, SE had higher cytotoxic potential with an  $IC_{50}$  of far below its  $IC_{50}$  of FRSA. In the case of SE with considerably low FRSA,

antioxidant/pro-oxidant effect seems to be less important. So, high cytotoxic potential may be attributed to corchorusins which are abundant in seeds of *Corchorus* sp. (Khan et al., 2006), having structural similarity with saikosaponins (Hsu et al., 2000; Mahato & Pal, 1987). Saikosaponins and saikosaponin-like compounds have been reported to possess potent antitumor activity (Bachran et al., 2008). In a study of Mallick et al. (2010), methanol extract of *Corchorus acutangulus*, its *n*-butanol fraction, and corchorusin-D inhibited cell growth and produced significant cytotoxicity in leukemic cell lines U937 and HL-60 via mitochondrial apoptotic pathway.

Depending on the extracted tissue, cytotoxic and genotoxic effects may vary. The  $IC_{50}$ , the concentration of a toxic substance at which the half viability is attained relative to control, is a useful parameter when selecting the test concentrations. In both half of  $IC_{50}$  LE and SE applied groups, DNA damage values increased ~10 folds. DNA damage increased ~17-fold with respect to control when extract concentrations were doubled. Doubling the extract concentration resulted in significant increase in DNA damage in nuclei of ARH-77 cells demonstrating genotoxicity of the extracts. As stated above, compounds with high antioxidant activity may exhibit pro-oxidant behavior, and pro-oxidant

activity can accelerate damage to molecules such as DNA, carbohydrates, or proteins (Aruoma et al., 1997). We have previously demonstrated that both ascorbic acid and  $\beta$ -carotene induce concentration and time-dependent genotoxic and cytotoxic damage on HepG2 human hepatocellular carcinoma cells together with increased oxidative damage at their relevant achievable plasma level concentrations (Yurtcu et al., 2011) in contrast to their protective effect on lymphocytes (Yurtcu et al., 2012). In addition, many compounds of plant origin may directly and irreversibly bind/damage to proteins involved in DNA replication, repair, and transcription, leading to damage at nucleic acid level. DNA damage observed in nuclei of ARH-77 cells might have been induced by direct or indirect acting of extract components on nucleic acids, and these deleterious effects may show variation depending on the extract component of different plant parts.

## Conclusions

In the present study, we demonstrated the *in vitro* cytotoxic effect of *C. olitorius* LE and SE on ARH-77 cells, with relation to TPC and FRSA of the extracts. The LE had high TPC and FRSA, and cytotoxic  $IC_{50}$  on ARH-77 cells above its  $IC_{50}$  for FRSA. On the other hand, SE had lower TPC and FRSA in comparison to LE but highly cytotoxic to ARH-77 cells. Both LE and SE induce genotoxic damage at relevant cytotoxic concentrations. *In vitro* cytotoxic effect and genotoxicity of LE may be attributed to pro-oxidant effect of phenol compounds, whereas cytotoxic potential of SE may be related to direct effect of bioactive constituents. Although more studies with other cancer cell lines are required, this study gives a preliminary insight to further research at molecular level and *in vivo* research on *C. olitorius*. To our knowledge, this is the first report demonstrating the high cytotoxic potential of *C. olitorius* SE and the genotoxic potential of LE and SE.

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## Declaration of interest

The authors report no declarations of interest. This study was supported by Baskent University Research Fund.

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