

Research Article



Cytotoxic Activity of Zinc Oxide Nanoparticles Using *Zingiber officinale* Rhizome Extracts against Human Ovarian Cancer (SKOV-3) Cell Line

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ABSTRACT

Zinc Oxide (ZnO) nanoparticle was synthesized using rhizome extracts of *Zingiber officinale* for in-vitro anti-cancer study in our previous paper. Treated with ZnO nanoparticle with *Zingiber officinale* extracts (ZGNPE) showed significant inhibition on the human chronic myeloid leukemia cells whereas in normal human embryonic kidney cells showed insignificant toxicity. Therefore, further more we evaluate the cytotoxic activity of ZnO nanoparticle with *Zingiber officinale* extracts on human ovarian cancer cells (SKOV-3). Cytotoxic activity was performed by MTT assay. Treated with ZGNPE sample 4,5 and 6 showed significant inhibition on the SKOV-3 cancer cells. The IC50 values were 48.49 µg for ZGNP-4, 39.83µg for ZGNP-5 and 45.36 µg for ZGNP-6 respectively. Thus, ZGNPE may be used as chemotherapy drug and an enormous medical application with less toxicity in future. The characterization of nanoparticle was confirmed using ultraviolet spectroscopy and Fourier Transfer Infrared Spectroscopy (FTIR). The ZGNPE characterization also used the UV-Vis Spectrophotometer spectrum technique to measure the structural characterization of nanoparticles by determine the absorbance measurement.

Keywords: Zinc Oxide Nanoparticles, *Zingiber officinale*, Ovarian Cancer, Cytotoxicity.

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INTRODUCTION

Nanoparticle represents a new platform to produce new novel cancer therapeutics.¹ Nanomaterials attracted interest due to the unique physical and chemical properties, and they have been used for a broad range of biomedical applications. However, in the recent year's transition metal oxide nanoparticles having semiconductor properties like ZnO, CuO, TiO₂ have attracted researchers to focus on their synthesis. Of these ZnO nanoparticle production has been concentrated more due to its diversifying properties which includes catalysis, electrical conductivity, antibacterial activity, cytotoxicity etc. Though there are several conventional physical and chemical methods available for their synthesis, biological method has attained a growing interest due to their advantages. The biological method of synthesizing metal oxide nanoparticles may be of three ways, which includes the use of microbes, plant or with enzymes. The use of plants or their extracts, for the synthesis of nanoparticles has been focused due to its simplicity, lower cost and also it involves proteins as capping or reducing agent. *Zingiber officinale* commonly known as ginger variety

pharmacological properties of such as anti-inflammatory, analgesic, anti-oxidant, hepatoprotective, which is due to the presence of volatile oils like sesquiterpene alcohol, zingiberol and monoterpene.² Over here *Zingiber officinale* extract was co-operated with ZnO nanoparticles in the form of precursor which will help to show the increase in cytotoxic activity.³ Cancer cells are normally highly-specialized cells which have regressed to a much simpler, more primitive stage and which, unlike the normal parent, divide continuously, although inefficiently.^{4,5,6} The mechanisms by which nanoparticles exerts their toxic effects are not well known, but some recent studies attributed it to their greater surface, permeability into cells, and accumulate within cells and organisms, as well as, membrane damage, inflammation, DNA damage, apoptosis, and change in interactions between cells to cells and matrix. In recent years, zinc oxide has promoted itself as an interesting metal oxide material because of its unique physical and chemical properties such as high chemical and mechanical stability, broad range of radiation absorption, high catalysis activity, electro chemical coupling coefficient, non-toxic nature etc.⁷ Zinc oxide can be synthesized using many different methods including micro emulsion synthesis, spray drying, sol-gel method, pyrolysis, controlled precipitation, RF plasma synthesis, vapor transport process etc. controlled precipitation method was used here for the synthesis of zinc oxide nanoparticles.⁸ In this study, we utilized zinc oxide nanoparticles using precursor of *Zingiber officinale* synthesized by Green synthesis method with the characterization of ZGNPE was done and we checked the effect of different ZGNPE samples on ovarian cell line.



MATERIALS AND METHODS

Chemicals and Reagents

Zinc Sulphate Heptahydrate (LobaCheme Pvt. Ltd.), Sodium Hydroxide LR (MERCK), D-MEM, HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), Fetal bovine serum (FBS), Penicillin-Streptomycin, Gentamycin, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), DMSO (dimethyl sulfoxide), Methanol, and the remaining chemicals and solvents were purchased from local firms and were of highest purity grade.

Cell Culture

SKOV-3 cells were purchased from National Centre of Cell Science, Pune. Human Ovarian cancer *cell line* SKOV-3 was used for *in-vitro* studies. The cells were sub-cultured as per the requirement of the experiment at an initial concentration of 1×10^6 cells/mL and SKOV-3 maintained in sterile RPMI 1640 medium supplemented with 10% heat inactivated FBS. Cultures were maintained at 37°C in a humidified atmosphere containing 5% CO₂ in air.

Preparation of ZnO Solution

Prepared different concentration like 0.25M, 0.5M and 1M of Zinc Sulphate Heptahydrate with weights taken like 3.59g, 7.18g and 14.37 gm. The solutions are dissolved in 100 mL distilled water. Kept over a magnetic stirrer and stayed for stirring almost for 10-15 min till clear solution comes.^{9,10}

Zingiber officinale extract preparation

2g of ginger was weighed and grinded in a mortar pestle. Ginger should be grinded well and paste was made. In a beaker, 50 mL of distilled water was taken and the paste was poured on it. At 70°C, it was kept for heating at least for 2hrs till the solution becomes yellow in colour. The yellow coloured solution was filtered with Whatman filter paper and the solution was kept in freeze at 4°C.¹¹

Green Synthesis- Preparation of ZGNPE

Different concentrations of prepared ZGNPE were prepared. On the prepared Zinc solution, slowly ginger extracted solution was added and kept under constant stirring. The temperature was maintained at 70°C in a magnetic stirrer and kept for stirring for at least 2-3hrs by keeping a petri dish over it. Maintaining pH at 12, NaOH was added in solution. After terminating the reaction, the pale yellow white ppt was then taken out and washed over and over again with distilled water followed by ethanol to get free of the impurities and then a pale-yellow powder of ZGNPE was obtained. The resulting powder was annealed at 400°C for 2hrs.^{1,12,13,14}

Characterization of ZGNPE

ZGNPE characterized was done by UV spectroscopy and FTIR (Fourier Transfer Infrared Spectroscopy). In UV, the peak was found at 364 nm. In FTIR, the absorption bands were found between 1500-1650 cm⁻¹ for C=C and C=N and 2000-2500 cm⁻¹ bands for alkynes. We confirmed that the

carbonyl groups from the amino acids residues and proteins have the stronger ability to bind metal indicating that the proteins could possibly from the metal nanoparticles to prevent agglomeration and thereby stabilize the medium. This suggests that the biological molecules could possibly thereby stabilize the medium. This suggests that the biological molecules could possibly perform dual functions of formation and stabilization of ZGNPE. This interaction makes these NPs a perfect candidate for various applications such as drug delivery¹⁵ and study of biological interactions.¹⁶

Cytotoxicity Study on SKOV-3 cell line

For cytotoxicity analysis SKOV-3 cells were seeded in 96 well tissue culture plate. They were separately treated with freshly prepared 1mg/ mL stock solution of ZGNPE in various concentrations 25, 50, 100 and 200 µg/ml at 24 hrs and incubated at 37°C in a humidified atmosphere containing 5% CO₂ in air. Untreated cells served as control. At the end of treatment, 20µL of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide] was added to each well and incubated for another 4 hrs at 37°C in a CO₂ incubator. The MTT assay is colorimetric assays for measuring the activity of enzymes that reduce MTT to formazan dyes, giving a purple colour. A solubilisation solution of DMSO 100µL is added in the insoluble purple formazan product into a coloured solution. The absorbance was taken at 570 nm SKOV-3 cells by micro plate manager (Reader type- Model 680 XR Bio-Rad laboratories Inc.). The IC₅₀ values were noted for both the cells.

Statistical Analysis

Percentage of cell growth inhibition was calculated by the following formula: % Cell Inhibition = $10 \times \frac{(O.D \text{ of Control} - O.D \text{ of treated})}{O.D \text{ of Control}}$, O. D=Optical Density.

Percentage of cell viability was calculated as follows: Viable Cells (%) = $\frac{\text{Total number viable cells per ml}}{\text{Total number of cells per 1ml}} \times 100$.

RESULTS

Zingiber officinale extract preparation

When the solution of well grind ginger is heated at 70°C, and the filtration is done with Whatman Filter Paper. The remaining solution was at 15-20ml.

Green Synthesis- Preparation of ZGNPE

The total yield percentage of all remaining extractions are here tabulated below. Yield % = $\frac{\text{Actual Yield}}{\text{Theoretical Yield}} \times 100\%$.

Identification and assay of Zn Salt

It becomes yellow when strongly heated, the yellow colour disappears on cooling. As the indicator is poured in dropwise, the solution becomes yellow. 1ml of 0.1M disodium edetate equivalent to 0.008138 gm of ZnO.



Table 1: % of Yield samples and pH of samples solution was maintained in alkaline state

Samples	Weight /gm	Yield samples /gm	Yield %
ZGNPE 1	3.59gm	2.54	70.75%
ZGNPE 2	3.59gm	2.34	65.18%
ZGNPE 3	3.59gm	2.54	70.75%
ZGNPE 4	7.18gm	6.54	91.08%
ZGNPE 5	7.18gm	5.87	81.75%
ZGNPE 6	7.18gm	5.91	82.31%
ZGNPE 7	7.18gm	5.74	79.94%
ZGNPE 8	14.37gm	12.84	89.35%
ZGNPE 9	14.37gm	12.45	86.63%
ZGNPE 10	14.37gm	11.46	79.74%

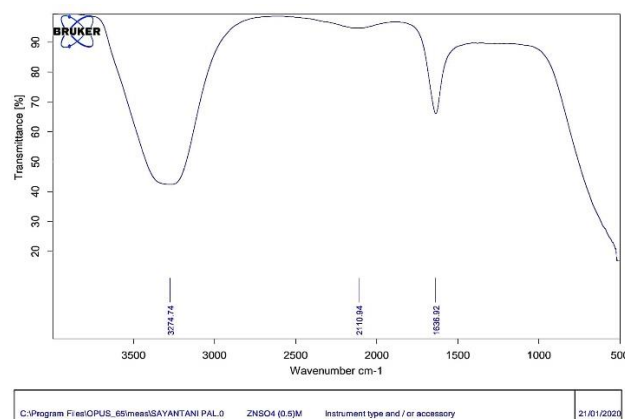
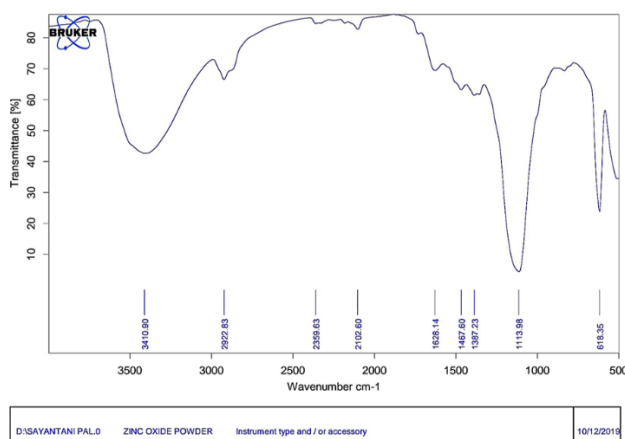
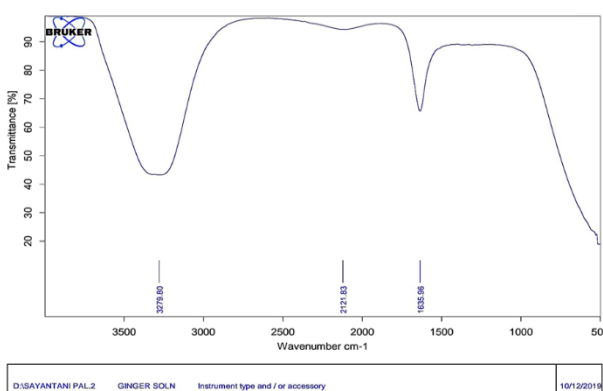
Table 2: The pH of ZGNPE Samples

Samples	pH
ZGNPE 1	12.41
ZGNPE 2	11.48
ZGNPE 3	11.25
ZGNPE 4	12.03
ZGNPE 5	12.01
ZGNPE 6	12.00
ZGNPE 7	12.08
ZGNPE 8	11.54
ZGNPE 9	11.67
ZGNPE 10	11.87

Fourier Transform Infrared Spectroscopy (FTIR)

All the apprehensible functional groups involved in capping and reduction of ZnO NPs were identified using FTIR (Bruker V70). FTIR measurements were carried out to identify the biomolecules for capping and efficient stabilization of the metal nanoparticles synthesized. From the analysis of FTIR studies we confirmed that the carbonyl groups from the amino acids residues and proteins has the stronger ability to bind metal indicating that the proteins could possibly from the metal nanoparticles to prevent agglomeration and thereby stabilize the medium. This suggests that the biological molecules could possibly perform dual functions of formation and stabilization of ZGNPE. Fig 10. image of ZGNPE showed the peak at bands between 1500 -1650 cm^{-1} for C=C and C=N and 2000-2500 cm^{-1} bands for alkynes. For Fig 11, the peak at 451 cm^{-1} in the characteristic absorption of Zn-O bond. Other absorption peaks while corresponding the carboxylate and hydroxyl impurities in the materials. Fig 12, from ginger extract solution, the absorption bands are found peak between

1635-2121 cm^{-1} . Aldehydes, ketones, carboxylic acids and esters were present between these bands.

**Figure 1:** FTIR reading of ZGNPE concentration of 0.5M. The absorption bands were found between 1500 -1650 cm^{-1} for C=C and C=N and 2000-2500 cm^{-1} bands for alkynes.**Figure 2:** FTIR reading of ZnO powder. The peak at 451 cm^{-1} in the characteristic absorption of Zn-O bond.**Figure 3:** FTIR reading of Ginger extract. The absorption bands are found peak between 1635-2121 cm^{-1} . Aldehydes, ketones, carboxylic acids and esters were present between these bands.

Cytotoxicity study on SKOV-3 cell line by MTT Assay

ZGNPE inhibited the growth and produced significant cytotoxicity of human ovarian cancer cell lines in a concentration-dependent manner. Reduction in the mean

OD of cells treated with the increasing dose of ZGNPE was observed as compared to control. ZGNPE exerted 50% growth inhibition (IC_{50}) of SKOV-3 cell line at concentration 25 μ g, 50 μ g, 100 μ g and 200 μ g for 24 hrs. The extract number four, five & six (ZGNPE) IC_{50} values were shown under in the time at 24 hrs and remaining extracts were

found insignificant cytotoxicity on cancer cell line. Remaining ZGNPEs exerted not above the 50% growth inhibition (IC_{50}) of cells at the concentrations 50 μ g, 100 μ g and 200 μ g for 24hrs. Toxicity effect does not observe on these results so further checking was not done.

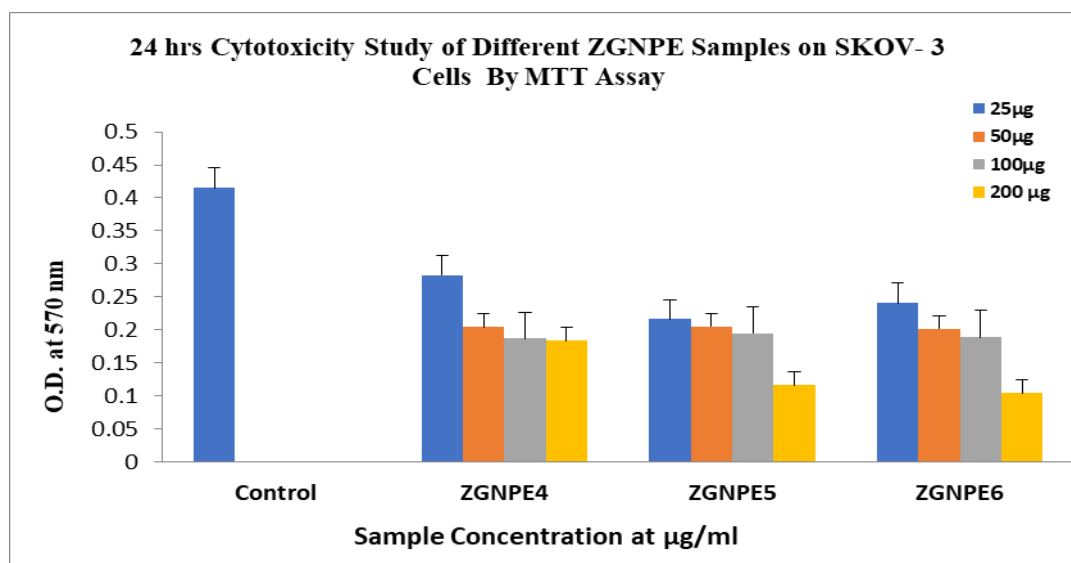


Figure 4: Histograms showed the OD value of SKOV-V cells after 24hrs of treatment with ZGNPE samples 4,5 and 6 respectively. The change in O.D. value 570nm for SKOV cells was observed against different samples. The % inhibition was calculated from the O.D. value. Data are mean \pm S.E.M.

Table 3: IC_{50} Values of ZGNPE on SKOV-3 Cells for 24 hrs

Samples	Time taken to reach 50%	IC_{50} values
ZGNPE 4	24 hrs	48.49 μ g/ml
ZGNPE 5	24 hrs	39.83 μ g/ml
ZGNPE 6	24 hrs	45.36 μ g/ml

DISCUSSION

Green synthesized zinc oxide nanoparticles appear in different shapes such as nano-powder or nano-cluster or nano-crystal the plant extract phenol compounds it may be effect on size and shapes.¹⁷ Green synthesized ZnO is an environmentally eco-friendly material, which is desirable especially for bio-applications, such as seed germination, bio-imaging and cancer detection. The use of zinc oxide nanoparticles is increasing in agricultural products and consumer goods, as well as several research works related to development of anti-cancer nanoparticulate dosage form. These type of application of biosynthesized ZGNPE have been under current research scenario by several workers.¹⁸ Which prompts the present research initiative.¹⁹ ZGNPE was synthesized using rhizome extracts of *Zingiber officinale* for *in-vitro* anti-cancer study supporting the latest findings of other biosynthesized zinc oxide nanoparticles for *in-vitro* cancer evaluations. Identification of zinc oxide has been done where results were shown zinc metal is present. Zinc oxide has been identified through pharmacopeial method of analysis (IP-2011, Vol- III, pg-2336) indicating the formation of ZGNPE. The ZGNPE characterization also used

the UV-Vis spectrum and FTIR technique to assess the structural characterization of nanoparticles. The UV-Vis spectra of synthesized nanoparticles were recorded on Shimadzu UV-1601 at 364nm operated at a resolution of 20 nm. Through FTIR the presence of zinc is confirmed through wave numbers between 500-2500 cm^{-1} .²⁰ These functional groups identify the formation of zinc oxide nanoparticles in the similar way as other researchers characterized the similar formation of biosynthesized ZGNPE as the functional groups associated with the ZGNPE formation having spectral peaks at 683–500 cm^{-1} and 698–505 cm^{-1} which proposed the formation of ZnO nanoparticles in *Zingiber officinale* extracts.²¹ ZGNPE concentration of 0.5M showed the peak at bands between 1500 -1650 cm^{-1} for C=C and C=N and 2000-2500 cm^{-1} bands for alkynes. For Zinc oxide KBr compressed pellets and found at the peak at 451 cm^{-1} in the characteristic absorption of Zn-O bond.²² Other absorption peaks while corresponding the carboxylate and hydroxyl impurities in the materials. Aldehydes, ketones, carboxylic acids and esters were present between these bands.²³ From ginger extract solution, the absorption bands are found peak between 1635-2121 cm^{-1} .²⁴ FTIR spectra affirm successful capping of biomolecules on the NP surface and thus provide stability and dispersion capacity to ZnO-NPs in aqueous media. Several studies have suggested an increase in *in vitro* cytotoxicity with nanophase ZnO compared with micrometer sized ZnO for several types of cancer, including glioma, breast, bone, ovarian, colon, and leukemias and lymphoma.²⁵ Cytotoxic activity was performed with varying concentrations (25 μ g, 50 μ g, 100

μg and $200 \mu\text{g/ml}$) against human ovarian cancer *cell line*, SKOV-3 cells. The change in O.D. value at 570 nm for SKOV-3 cells was observed against different samples. The % inhibition was calculated from the O.D. value. In SKOV-3 cells different ZGNPE samples inhibited the cell growth and produced significant cytotoxicity in a concentration-dependent manner. A number of other researchers have noticed that Zinc oxide nanoparticles are cytotoxic only to cancerous cells and do not have any toxic effects on normal cell lines.^{26,27} However, the cytotoxicity of some chemical synthesized nanoparticles has been confirmed on normal cells even at lower concentration in comparison of cancer cell lines; for this reason, green synthesis of nanoparticles becomes more attractive as an alternative method due to the decreasing bio toxicity effects of nanoparticles, through the use of non-noxious components instead of chemicals agent over the last recent years.²⁸ It was also reported that morphological changes induced by cytotoxic ZGNPEs on different cell lines. Morphological changes in cells may affect their metastatic process including substrate attachment, migration and invasion.²⁹ On HEK293T cells, showed minimal toxic effect on applying various concentrations ($25 \mu\text{g}$, $50 \mu\text{g}$, $100 \mu\text{g}$ and $200 \mu\text{g/ml}$) at 24hrs in our previous study. The cytotoxic activity yielded inhibition of concentration at $48.49 \mu\text{g}$ for ZGNP-4, $39.83 \mu\text{g}$ for ZGNP-5 and $45.36 \mu\text{g}$ for ZGNP-6 respectively. ZGNPE sample 4,5 and 6 are highly cytotoxic to SKOV-3 cells and the solubilization of ZnO is the main toxicological mechanism. Based on enhanced biocompatibility, improved solubility and less toxicity, the efficacy of ZGNPEs synthesized from *Zingiber officinale* in the field of medicine could play a significant role in future. In the light of current research, ZGNPEs could be developed as a potential next generation cancer treatment strategy. In order to do so, further research must be carried out in order to resolve the issue of cancer, a long-term health hazard.

CONCLUSION

The present study dealt with the biosynthesis of ZGNPE using the root extracts of *Zingiber officinale* that were characterized using UV Visible spectroscopy and FTIR. These results reveal that the nanoparticle were spherical in shape and had an average size. In UV, peak was found at range of 364 nm. FTIR bands were shown through FTIR it is confirmed that zinc metal is present where wavelength is shown at $500\text{-}2500 \text{ cm}^{-1}$. ZGNPE concentration of 0.5M showed the peak at bands between $1500\text{-}1650 \text{ cm}^{-1}$ for C=C and C=N and $2000\text{-}2500 \text{ cm}^{-1}$ bands for alkynes. For Zinc oxide solution where compressed with KBr pellets and found at the peak at 451 cm^{-1} in the characteristic absorption of Zn-O bond. Other absorption peaks while corresponding the carboxylate and hydroxyl impurities in the materials. From ginger extract solution, the absorption bands are found peak between $1635\text{-}2121 \text{ cm}^{-1}$. Aldehydes, ketones, carboxylic acids and esters were present between these bands. The synthesized ZGNPE different samples were checked for cytotoxic activity on SKOV-3 and HEK293T cells which showed some desirable results on which we could conclude that sample on various

concentrations are achieved with our motives. Moreover, the size of the nanoparticle also influenced the nanoparticle's cytotoxic activity. The ZGNPE shows higher activity against SKOV-3 cell line. The % inhibition almost showed 50% with in 24hrs. In HEK293T cell line, there was no toxic nature found for the samples. Thus, we can say it is non-toxic in nature. ZGNPE are highly cytotoxic to SKOV-3 cells and the solubilization of ZnO is the main toxicological mechanism. The transition from oncogenomics to innovative mechanism-based cancer therapeutics is now well underway. Thus, by using the greener method for producing Zinc Oxide Ginger Nanoparticle Extracts (ZGNPE) can have a potential activity on treating ailments pertaining to cytotoxicity and hence further study in this regard is required to establish if beneficial effects in human being.

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