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HEPATOPROTECTIVE ACTIVITY OF *BARLERIA CRISTATA* LINN. (WHOLE PLANT)

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Keywords:

Barleria Cristata Linn, Hepatoprotective activity, Ethanol extract, Petroleum ether, Cell survivability

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ABSTRACT: The present study evaluated the hepatoprotective potential of *Barleria cristata* extracts petroleum ether (BCL-PE) and ethanol (BCL-EE) against carbon tetrachloride (CCl_4)-induced toxicity in cultured Chang liver cells. CCl_4 is a known hepatotoxin that generates reactive oxygen species, causing oxidative damage and cell death in liver tissues. The cytotoxicity of CCl_4 was assessed using the MTT assay, which revealed an IC_{50} value of 62.5 µg/mL and a 49.41% reduction in cell viability. A dose-dependent decline in viability was observed, with the lowest level of 7.05% at 1000 µg/mL. To evaluate the protective effects, cells were pre-treated with various concentrations of BCL-PE and BCL-EE before CCl_4 exposure. Both extracts demonstrated a concentration-dependent increase in cell viability. At the lowest tested dose (7.2 µg/mL), BCL-PE and BCL-EE restored viability to 92.94% and 93.52%, respectively. Microscopic analysis supported these findings, showing improved cellular morphology in extract-treated groups compared to cells treated with CCl_4 alone. These results suggest that *Barleria cristata* possesses significant hepatoprotective properties, likely due to its antioxidant and cytoprotective constituents. The findings support its traditional use in liver-related ailments. Further research is needed to isolate key bioactive compounds and investigate their mechanisms *in-vivo*, potentially contributing to the development of novel plant-based liver therapeutics.

INTRODUCTION: The human liver, the second-largest organ, is responsible for a wide array of more than 5,000 essential bodily functions. These include blood clotting, detoxifying harmful substances from the bloodstream, converting food into essential nutrients, regulating hormone balance, combating infections and diseases, regenerating tissue after damage, and maintaining appropriate levels of cholesterol, glucose, and iron through metabolism.

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Liver disorders have emerged as a significant cause of illness and death in both humans and animals worldwide, with drug-induced liver toxicity being one of the leading contributing factors [1]. Among the various conditions that impact the liver, viral hepatitis is the most prevalent. It refers to liver inflammation triggered by a viral infection. However, hepatitis can also result from exposure to certain drugs, bacteria, toxic mushrooms, and parasites such as amoebas or Giardia.

Liver-related illnesses are responsible for approximately 20,000 deaths annually. Scientific studies in herbal medicine have highlighted its potential as an alternative treatment for liver diseases. As a result, global research efforts are increasingly directed toward identifying hepatoprotective agents derived from medicinal

plants [2]. Various traditional plant-based remedies from different parts of the world have been examined for their antioxidant potential and potential to protect the liver from damage in experimental studies [3]. These findings may contribute to the development of affordable liver-protective medications. The hepatoprotective activity of both drugs and herbal extracts is most frequently evaluated using animal models with Liver damage caused by carbon tetrachloride (CCl_4) [4, 5].

Barleria genus, part of the *Acanthaceae* family, thrives mainly in tropical zones of Asia and Africa, particularly within open forest ecosystems. Among the genera in this family, *Barleria* ranks third in terms of the number of species, comprising around 300. In India, Balkwill documented 32 species, while Karthikeyan and his team identified one subspecies, six varieties, and 29 distinct species [6, 7]. Despite its name, the "Philippine violet" is neither native to the Philippines nor related to true violets, although its blossoms are violet-colored. *Barleria cristata*, commonly grown for ornamental purposes, is a dense, evergreen subshrub with upright, hairy stems, typically reaching a height of 3 to 4 feet. The plant is now widely grown in various tropical and subtropical regions, including India, South China, and Southeast Asia. In the United States, it can be cultivated in Florida, southern Texas, Louisiana, Arizona, and California. However, this shrub is considered a potentially invasive species, particularly in disturbed areas and along roadsides [8, 9, 10]. The phytochemical composition of *Barleria cristata* includes triterpenoids, flavonoids, phenolic substances, iridoids and phenylethanoid glycosides [11, 12]. This plant has demonstrated a range of biological effects, such as liver-protective, antiplasmodial, antibacterial, antifungal, antidiabetic, anti-inflammatory, and antioxidant properties [13, 14, 15]. For this study, normal liver cell lines were used to evaluate the hepatoprotective activity of *Barleria cristata* using both petroleum ether and ethanolic extracts.

METHODOLOGY AND MATERIALS:

Gathering and Confirmation of Botanical Material: *Barleria cristata* Linn was gathered from a nearby herbal garden located in Kakapalayam. Plant specimen was authenticated and verified by Dr. K. N. Sunil Kumar, serving as a Research Officer at the Central Research Institute for Siddha, situated within the Anna Government Hospital Campus, Arumbakkam, Chennai.

Preparation and Processing of Plant Specimens: The leaves of the plant were gathered and thoroughly rinsed under the tap water to eliminate any dust and impurities, then rinsed with distilled water. The leaves were chopped into smaller segments and dried in the shade for a period of 7 to 10 days. The dehydrated material was finely milled using a mechanical device. The powdered sample was kept in a sealed container at room temperature until used for extraction.

Extraction Technique Applied to the Fine Powder: The dried and ground plant sample underwent sequential extraction using petroleum ether followed by ethanol, targeting the separation of compounds based on their differential solubility in each solvent. The obtained extracts were gathered, passed through filtration, and then concentrated using a rotary evaporator. The evaporated extract was designated and labelled as BCW-PE (petroleum ether extract) and BCW-ET (ethanol extract), respectively. These were subsequently stored in airtight glass containers within a desiccator for future analysis.

Pharmacological Activity:

Hepatoprotective Activity:

Cell Line and Culture Conditions: Normal Chang liver cells used in this experiment were obtained from the National Centre for Cell Science (NCCS), Pune. The cells were cultured in Minimum Essential Medium (MEM) containing 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 µg/mL streptomycin. Cultures were performed at 37 °C in a humidified inhibitor 5% CO_2.

Reagents and Chemicals: Minimum Essential Medium (MEM) was purchased from HiMedia. Fetal bovine serum (FBS) was acquired from Cistron Laboratories. Trypsin, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl. tetrazolium bromide), and DMSO (dimethyl sulfoxide) were sourced from Sisco Research Laboratories, Mumbai. All additional reagents and chemicals employed in the study were procured from Sigma-Aldrich, Mumbai.

***In-vitro* Assessment of Cytotoxic and Hepatoprotective Effects Using the MTT Assay**

Cytotoxic effects of carbon tetrachloride (CCl_4) on Chang liver cells was analyzed through the MTT method as outlined by Mosmann *et al.,* (1983). A cell suspension with a concentration of 1×10^5 cells per well was dispensed into 6-well plates (Costar Corning, Rochester, NY) with 5 mL of culture medium in each well. After 48 hours of growth, the cells reached confluence. They were then exposed to the test compounds and maintained at 37 °C for another 24 to 48 hours. At the end of this period, the medium containing the test compounds was discarded, and the wells were carefully washed using phosphate-buffered saline (PBS), adjusted to pH 7.4. 1 mL of 0.5% MTT reagent (5 mg/mL prepared in PBS) was introduced into each well. After an incubation period of 4 hours, formazan crystals formed by viable cells were dissolved using isopropanol mixed with 0.04 M hydrochloric acid. Cell viability was quantified by reading the absorbance at 570 nm using a spectrophotometer.

Hepatoprotective Activity Assay: To assess hepatoprotective potential, Chang liver cells were first exposed to carbon tetrachloride (CCl_4) to induce cellular damage. The CCl_4-injured cells were then treated with two different extracts BCL-PE (petroleum ether extract) and BCL-EE (ethanolic extract) to evaluate their protective effects on liver cells.

Following treatment, cell viability was measured, and the concentration required to inhibit 50% of cell viability (IC_{50}) was determined from the dose-response curve. Absorbance was recorded at 570 nm using a UV spectrophotometer, with untreated wells containing only cells and no sample serving as the blank control.

The impact of the extracts on Chang liver cell proliferation was represented as a percentage of cell viability, calculated using the formula:

$$\% \text{ Cell Viability} = (A_{570} \text{ of Treated Cells} / A_{570} \text{ of Control Cells}) \times 100$$

Test Samples Used:

BCL-PE: (Petroleum Ether Extract)

BCL-EE: (Ethanolic Extract)

Carbon Tetrachloride: (CCl_4)

RESULTS AND DISCUSSION:

TABLE 1: IMPACT OF CCL_4 ON THE SURVIVAL RATE OF CHANG HEPATIC CELL LINE

Entry no.	Dosage (µg/ml)	Absorbance (optical density)	Cell survival rate (percentage)
01	1000	0.06	7.05%
02	500	0.16	18.82%
03	250	0.29	34.11%
04	125	0.34	40.00%
05	62.5	0.42	49.41%
06	31.2	0.49	57.64%
07	15.6	0.53	62.35%
08	7.2	0.62	72.94%
09	Cell control	0.85	Complete Cell Survival

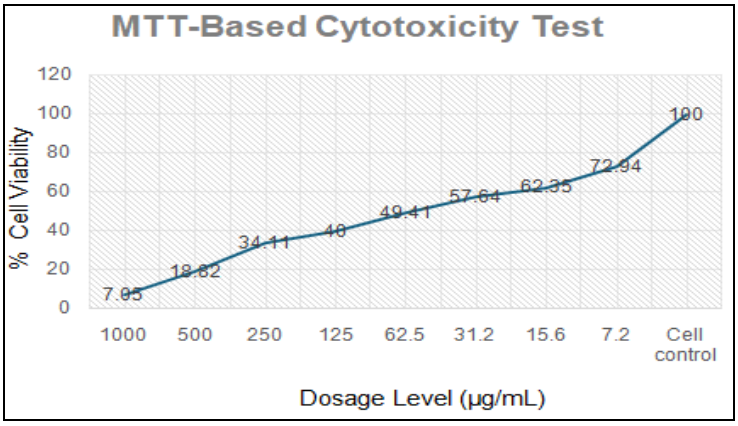

FIG. 1: IMPACT OF CCL_4 ON THE SURVIVAL RATE OF CHANG HEPATIC CELL LINE

TABLE 2: EVALUATION OF BCL PE'S PROTECTIVE ACTIVITY AGAINST CCL_4-INDUCED LIVER CELL TOXICITY

Entry no.	Dosage (µg/ml)	Absorbance (optical density)	Cell survival rate (percentage)
01	1000 (µg/ml)	0.47	55.29%
02	500 (µg/ml)	0.52	61.17%
03	250 (µg/ml)	0.58	68.23%
04	125 (µg/ml)	0.61	71.76%
05	62.5 (µg/ml)	0.66	77.64%
06	31.2 (µg/ml)	0.71	83.52%
07	15.6 (µg/ml)	0.76	89.41%
08	7.2 (µg/ml)	0.79	92.94%
09	Control Cells	0.85	100%

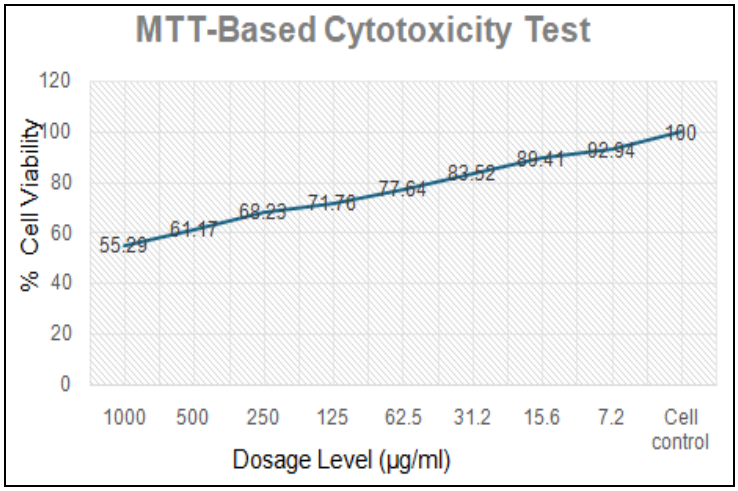

FIG. 2: BCL PE'S PROTECTIVE ACTIVITY AGAINST CCL_4-INDUCED LIVER CELL TOXICITY

TABLE 3: LIVER-PROTECTIVE ACTIVITY OF BCL EE IN CCL_4-TREATED CHANG HEPATIC CELL LINE

Entry no.	Dosage (µg/ml)	Absorbance (Optical density)	Cell survival rate (percentage)
01	1000 (µg/ml)	0.53	62.35%
02	500 (µg/ml)	0.60	7058%
03	250 (µg/ml)	0.68	80.00%
04	125 (µg/ml)	0.74	87.05%
05	62.5 (µg/ml)	0.79	92.94%
06	31.2 (µg/ml)	0.81	95.29%
07	15.6 (µg/ml)	0.83	97.64%
08	7.2 (µg/ml)	0.84	98.82%
09	Control cell	0.85	Completly viable

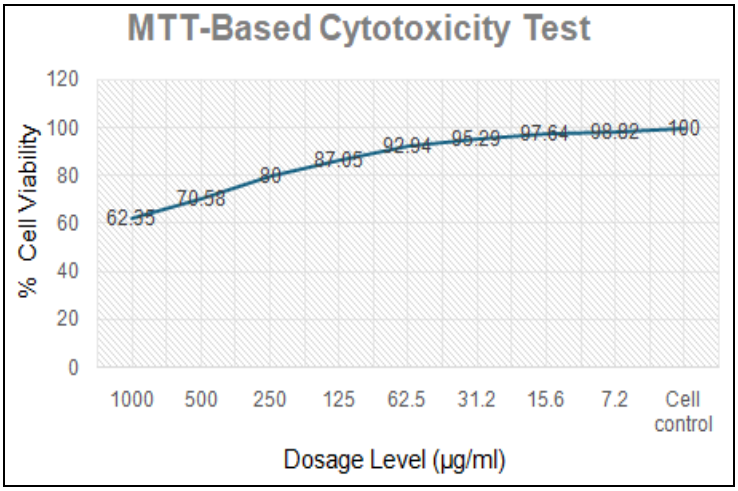

FIG. 3: LIVER-PROTECTIVE ACTIVITY OF BCL EE IN CCL_4-TREATED CHANG HEPATIC CELL LINE

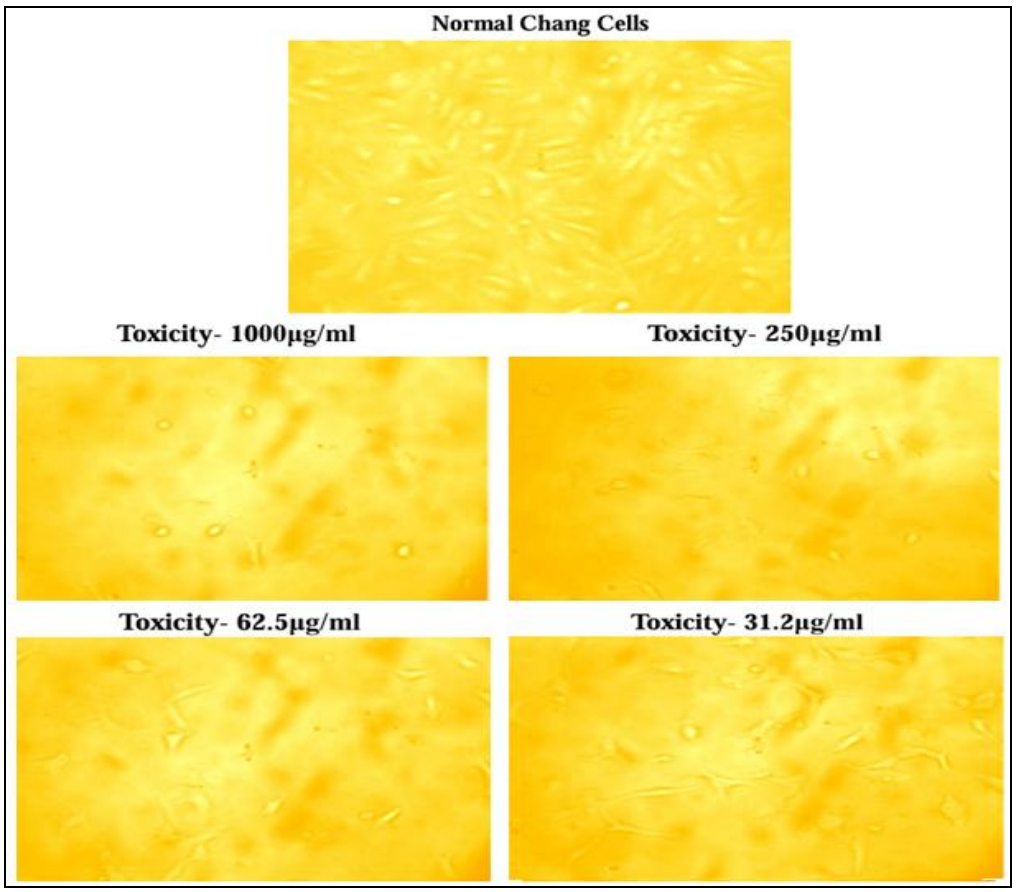

FIG. 4: EFFECT OF CCL_4 ON CELL VIABILITY IN CHANG LIVER CELL LINE

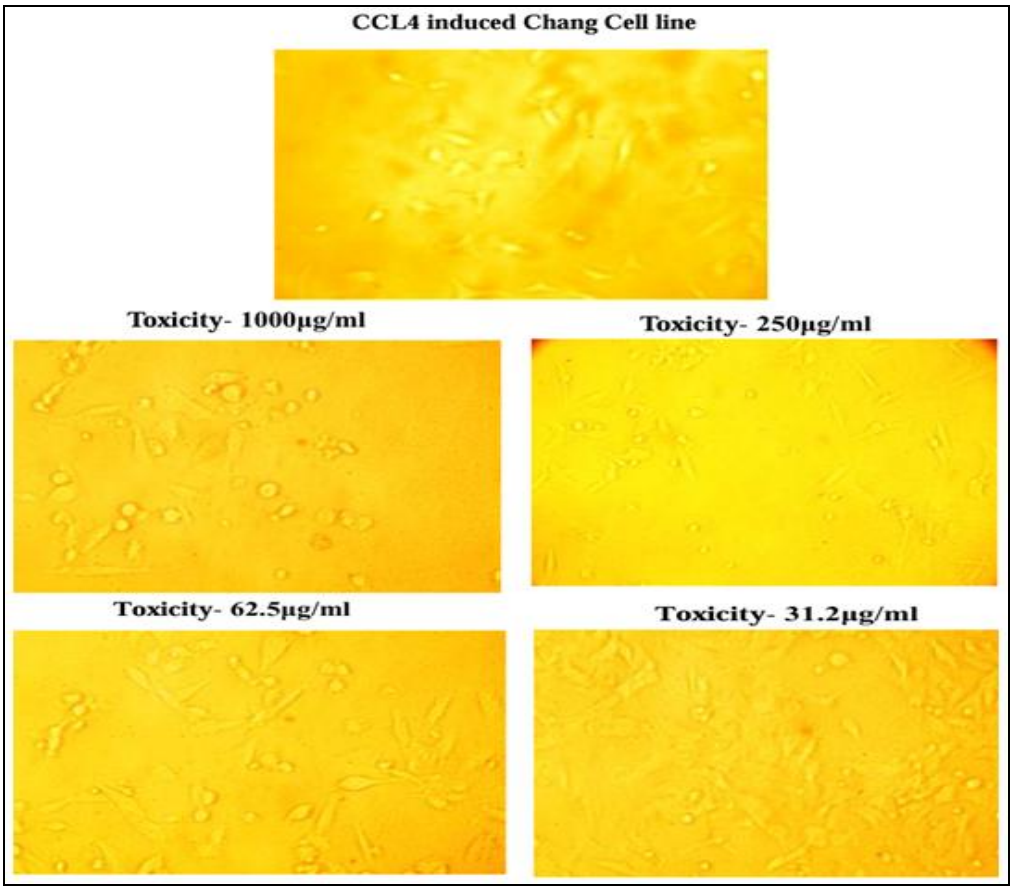

FIG. 5: PROTECTIVE EFFECT OF BCL PE ON CCL_4-INDUCED DAMAGE IN CHANG LIVER CELL LINE

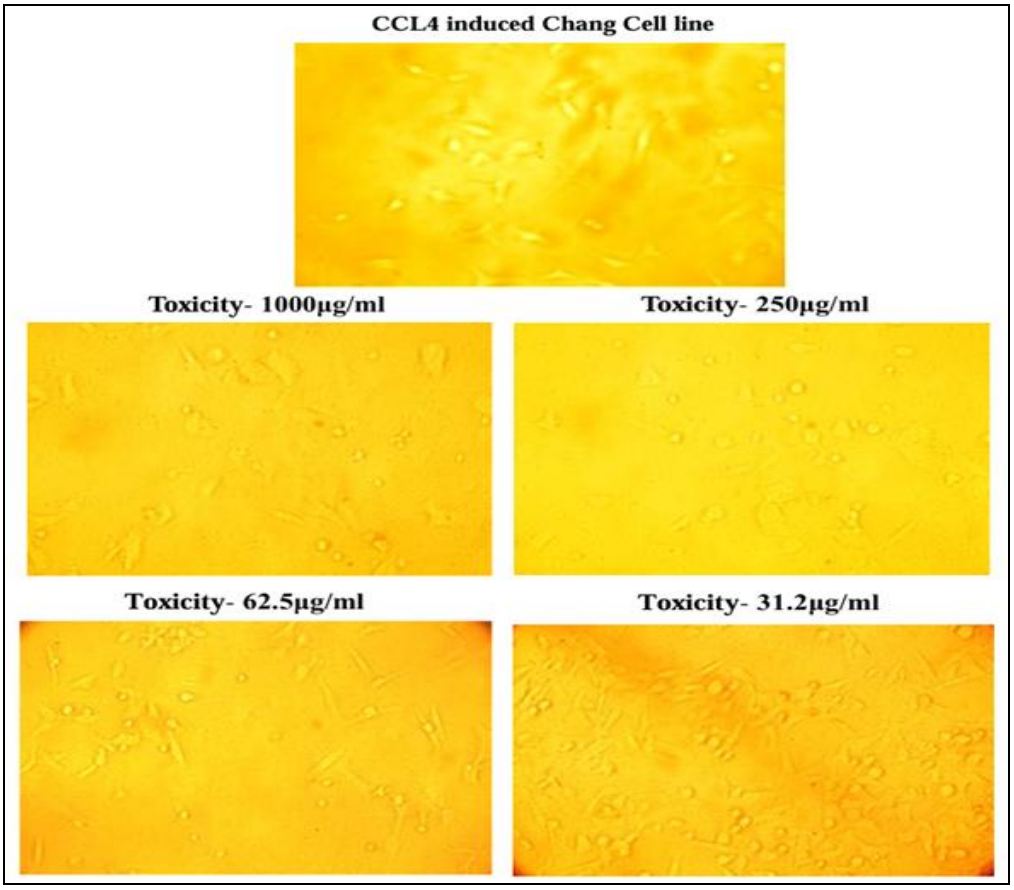

FIG. 6: HEPATOPROTECTIVE ACTIVITY OF BCL EE IN CCL_4-INDUCED CHANG LIVER CELL LINE

CONCLUSION: Upon investigating the cytotoxic effects of carbon tetrachloride (CCl_4) on Chang liver cell lines, a significant reduction in cell viability was noted dropping to 7.05% at a concentration of 1000 µg/Ml indicating strong hepatotoxicity. IC_{50} was estimated to be 49.41%, further validating the harmful impact of CCl_4 on liver cells.

Following this step, the liver-protective properties of *Barleria cristata* extracts, both ethanolic (BCL-EE) and petroleum ether (BCL-PE) were investigated using carbon tetrachloride-induced toxicity as a model. Both extracts showed a protective effect; however, the ethanolic extract (BCL-EE) demonstrated superior hepatoprotective potential, as reflected by consistently higher cell viability across all tested doses.

These findings indicate that BCL-EE may offer more effective therapeutic benefits for liver protection when compared to BCL-PE.

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CONFLICTS OF INTEREST: This research was conducted without any declared conflicts of interest by the authors**.**

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