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ABSTRACT BOOK

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Portuguese Propolis enriched phenolic extract: Reactive Oxygen Scavenging and Cytoprotective Activities



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INTRODUCTION

Propolis, a resinous natural product produced by honeybees, is claimed to have a wide range of beneficial activities for human health which have been attributed to its phenolics [1]. In general, phenolics account for approximately half of propolis weight, although its content and composition can greatly vary with propolis geographical origin.

This study aims to determine the antioxidant and cytoprotective properties of Northeast Portuguese propolis.

METHODS

- The propolis hydroethanolic purified extract (PPE) was obtained by extraction at 70 °C/1 h and recovery onto SPE C₁₈ cartridges [2];
- Folin Ciocalteu method [3] was used for estimation of total phenolics amount;
- Main phenolics determination was carried out by HPLC/ESI-MSⁿ analysis [4];
- A dichromate potassium-stimulated (DK) HepG2 cell model was used to evaluated:
 - Reactive oxygen species (ROS) scavenging by flow cytometry, after incubation with DK for 48 h [5];
 - The cytoprotective properties by the MTT assay, after 1.5 μM and 500 μM of DK exposure for 72 h or 6 h, respectively [5].

RESULTS

AND

DISCUSSION

The total amount of phenolics in the PPE accounted for 375.4±5.8 mg GAE/g of extract and this was enriched in chrysin, pinocembrin and pinobanksin-3-O-acetate. Minor components include several phenolic acid derivatives

PPE decreased the rate of ROS production about 50% and exerted an effective protection against the reduction of cell viability of 9% and 22%, for HepG2 cells exposed to DK for 6 h and 72 h, respectively.

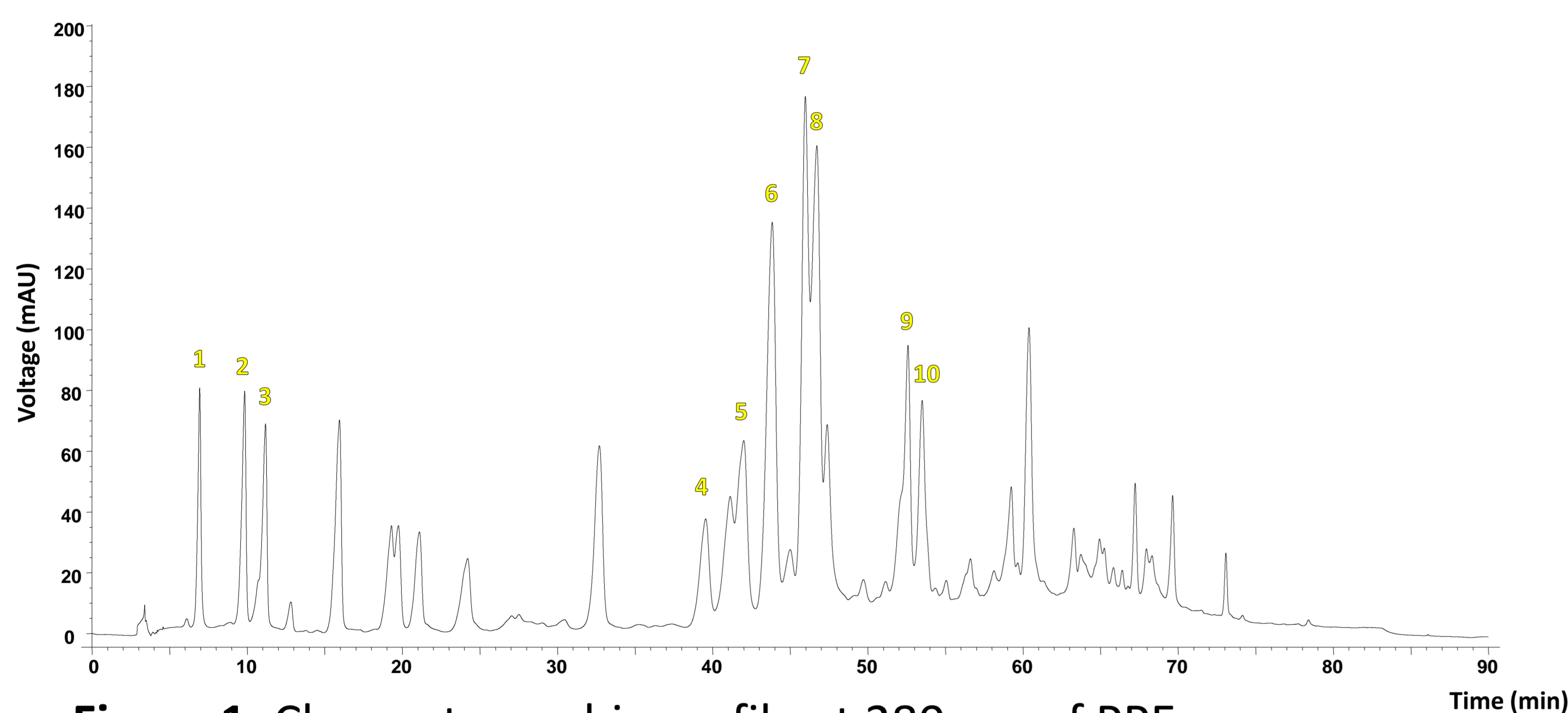


Figure 1. Chromatographic profile at 280 nm of PPE.

Table 1. ESI-MSⁿ data fragmentation of HPLC eluting fractions in PPE

| Peak | t _R (min) | [M-H] ⁻ (m/z) | HPLC/ESI-MS ⁿ | Compound |
|------|----------------------|--------------------------|--|---|
| 1 | 6.91 | 179 | MS ² [253]: 135, 163 | Caffeic acid |
| 2 | 9.8 | 163 | MS ² [163]: 119 | <i>p</i> -Coumaric acid |
| 3 | 11.5 | 193 | MS ² [193]: 177, 149, 133 | Ferulic acid |
| 4 | 39.5 | 247 | MS ² [247]: 179, 135 | Caffeic acid isoprenyl ester |
| 5 | 41.1 | 247 | MS ² [247]: 179, 135 | Caffeic acid isoprenyl ester isomer |
| | | 269 | MS ² [269]: 178, 134, 221, 265, 161 | Caffeic acid benzyl ester |
| 6 | 43.8 | 255 | MS ² [255]: 211, 213, 145, 151 | Pinocembrin |
| 7 | 45.9 | 253 | MS ² [253]: 151, 179, 249, 209 | Chrysin |
| 8 | 46.7 | 313 | MS ² [313]: 253 | Pinobanksin-3-O-acetate |
| | | 283 | MS ² [283]: 179, 135 | Caffeic acid phenylethyl ester |
| 9 | 51.9 | 231 | MS ² [231]: 163, 119 | <i>p</i> -coumaric acid isoprenyl ester |
| | | 295 | MS ² [295]: 178, 134, 251 | Caffeic acid cinnamyl ester |
| 10 | 53.5 | 403 | MS ² [403]: 255, 281, 267, 359 | Pinocembrin-5-O-3-hydroxy-4-methoxyphenylpropionate |
| | | 327 | MS ² [327]: 253, 271 | Pinobanksin-3-O-propionate |

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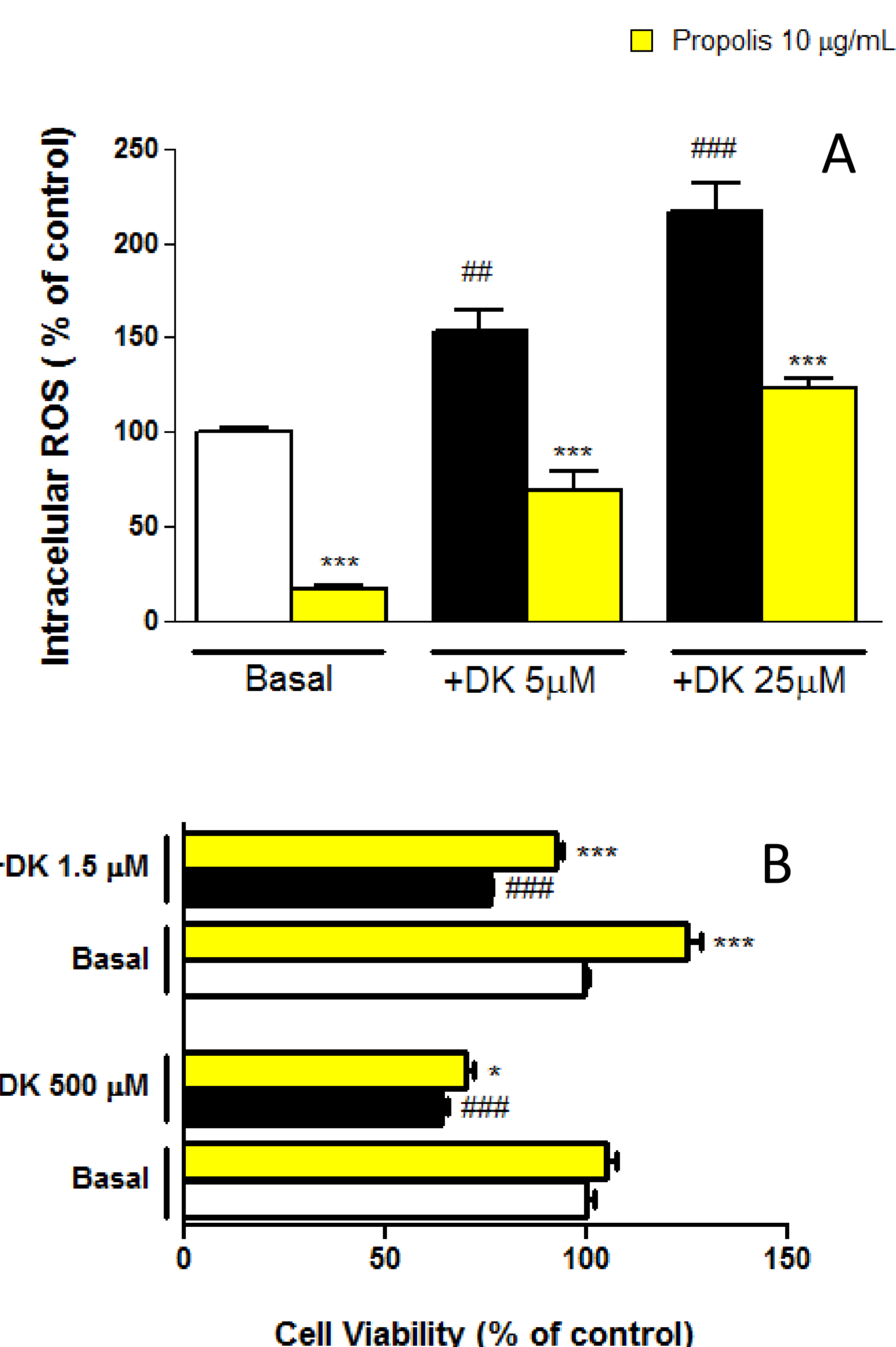


Figure 2.

Protective effects of the PPE against the DK-induced generation of ROS (A) and against cell viability decrement (B) in human hepatoblastoma HepG2 cells.

CONCLUSION

Overall the results emphasize important activities of PPE that can be related to the high content of phenolic compounds. To determine the exact contributions of each polyphenol, further studies should be performed.

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