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Beta vulgaris Ethanolic Root Extract: A Novel Cytoplasmic Dye for Tissue DemonstrationOkoye J.O.^{1,2*}, Ubani J.U.², Awwioro, O.G.³, Okechi, O.O.⁴, Benard, S.A.⁵

Department of Medical Laboratory Science, Faculty of Health Sciences and Technology, College of Medicine, Nnamdi Azikiwe University, Nnewi campus, Anambra State, Nigeria¹, Department of Medical Laboratory Science, School of Public and Allied Health, Babcock University, Ogun State², Faculty of Science, Delta State University, Abraka, Delta State³, Department of Medical Laboratory Science, Abia State University, Uturu, Abia State⁴, Histopathology Laboratory Unit, Department of Pathology, University of Ilorin Teaching Hospital, Ilorin, Kwara State⁵

Author for Correspondence*: +234-703-111-9994/judeogeokoye@gmail.com

Abstract

This experimental study evaluated the potential of *Beta vulgaris* root dye as a novel nuclear and cytoplasmic dye. Dyes were extracted from *B. vulgaris* root using 50% and 80% ethanol, and distilled water at pH 5.0 and 11.0. A total of 66 sections from liver, kidney and testis (22 each) were stained in the extracted dye and evaluated against tissues stained by conventional Haematoxylin and Eosin (H&E) technique. The result revealed that 50% *B. vulgaris* ethanol extract at a pH of 5.0 gave the best cytoplasmic staining when compared with other experimental dyes. Tissues stained in equal parts of *B. vulgaris* and Eosin gave excellent cytoplasmic staining when used as counter (secondary) dyes than conventional H&E technique. However, none of the experimental dyes differentially stained the nuclei in tissues. This study suggests that *B. vulgaris* ethanolic root extract could be used as a cytoplasmic dye in place of Eosin.

Keywords: *Beta vulgaris* root, Ethanol, Dye, Histology, Cytoplasm

Introduction

Substances with intense colors are often called colorants, which may be natural (for example, Haematoxylin) or synthetic (for example, Eosin). Colorants are classified into dyes and pigment depending on the ability of the substance to retain or lose their structure in a solvent. A pigment keeps its structure while a dye loses its structure when dissolved in a solvent (Nuffield Foundation, 2008). The use of natural dyes which are cheaper, eco-friendly and biodegradable has been advocated due to the

increased environmental awareness and need to avoid some hazardous effects of synthetic dyes such as Eosin (Eom *et al.*, 2001). An example of a plant used as food colorant is *Beta vulgaris* (Beet root). Beet root is dicotyledonous and herbaceous, and belongs to Chenopodiaceae family (Al-Almura *et al.*, 2012; Al-Kubaasy, 2004). The colour of the root bulb mainly comprises of red-violet-colored betacyanins (betanin, isobetanin, probetanin and neobetanin) and yellow-orange-colored betaxanthins (Singh and Hathan, 2014; Attia *et al.*, 2013). Red beet extract has been used in staining internal structures of intestinal parasites such as *Trichuris trichiura* and *Ascaris lumbricoides* in shades of yellow and red (Cheng *et al.*, 2014; Al-Almura *et al.*, 2012). The type of dye extracted is dependent on the pH, extracting solvent and mordant used (Cheng *et al.*, 2014; Suebkhamphet and Naimon, 2014; Samanta *et al.*, 2007; Okolie, 2006; Llewellyn, 2005). The multiplicity of stains in histology shows that available dyes are individually insufficient in laboratory investigations. Thus, this study explored the potential of some experimental methods in expressing the four colour-spectrum of *B. vulgaris* root extract.

Materials and Methods

Fresh roots of *B. vulgaris* were obtained from community market in Ilishan-Remo (Ogun State), identification and authentication by a taxonomist. Sections were obtained from formalin fixed and processed liver, kidney and testes of albino rats.

Study design and technique

This experimental study included 66 sections which were categorized into 22 groups (3 sections each) based on the staining techniques: Group 1 (conventional Haematoxylin and Eosin staining technique), Group 2 (Harris alone), Group 3 (Harris and 80% methanol *B. vulgaris* extract at pH 5.0), Group 4 (Harris and aqueous *B. vulgaris* extract at pH 5.0), Group 5 (Harris and 50% methanol *B. vulgaris* extract at pH 5.0), Group 6 (Harris and 80% methanol *B. vulgaris* at pH 5.0) and Group 7 (Harris and Equal parts of 80% methanol *B. vulgaris* extract and Eosin), Group 8 (Harris and equal parts of aqueous *B. vulgaris* extract and Eosin), Group 9 (Harris and equal parts of 50% methanol *B. vulgaris* extract and Eosin), Group 10 (50% methanol *B. vulgaris* extract at pH 5.0), Group 11 (80% methanol *B. vulgaris* extract at pH 5.0), Group 12 (Aqueous *B. vulgaris* extract at pH 11.0 with iron mordant and Eosin), Group 13 (Aqueous *B. vulgaris* extract at pH 11.0 with alum mordant and Eosin), Group 14 (80% methanol *B. vulgaris* extract at pH 11.0 with iron mordant and Eosin), Group 15 (80% methanol *B. vulgaris* extract [at pH 11.0 with alum mordant] and Eosin), Group 16 (80% methanol *B. vulgaris* extract [at pH 11.0] and Eosin), Group 17 (80% methanol *B. vulgaris* extract [at pH 5.0 with iron mordant] and Eosin), Group 18 (aqueous *B. vulgaris* extract [at pH 11.0] and Eosin), Group 19 (50% methanol *B. vulgaris* extract [at pH 11.0 with iron mordant] and Eosin), Group 20 (50% methanol *B. vulgaris* extract [at pH 11.0 with alum mordant] and Eosin), Group 21 (50% methanol *B. vulgaris* extract [at pH 11.0] and Eosin) and Group 22 (Eosin alone) (Avwioro, 2002).

Evaluation of stained tissues and cells

The stained tissue sections were evaluated by 3 specialists (Histopathologists) against sections stained by conventional H&E technique with respect to nuclear cytoplasm differentiation.

Ethical issue

This study was carried out in accordance with the

ethical guidelines of Babcock University Health Research Ethics committee (BUHREC 426/17).

Extraction

Meshed red beet (*Beta vulgaris L.*) was mixed with 100 ml of 50% ethanol, 80% ethanol and distilled water (10 g each). The mixtures were heated to 45 °C in a water bath for three hours and allowed to cool. The solutions were allowed to stand for 48 hours (at room temperature) before extraction. The extractions were carried out at different pH (5.0 and 11.0). The pH of the extract (4.83) were determined and adjusted using microprocessor pH meter (Hanna pH 211). Acidity was achieved using Hydrochloric acid (0.125 ml) while alkalinity was attained using Sodium hydroxide (0.125 ml). Extracts were purified accordingly. The extracting solvents were removed using Stuart's rotary evaporator (Model number: nxDS6i and serial number: R000103507). Few drops of stabilizer (Thymol) was added and the extracts were poured in amber bottles and stored at 4 °C until when needed (Cheng *et al.*, 2014; Francis, 2000),

Constituents of dye preparations:

1. Constituents of alum stain (20 ml): ethanol (20 ml), thymol (10 mg), extract (3.32 g) and mordant (2.0 g)
2. Constituents of iron stain (20 ml): ethanol (20 ml), Thymol (10 mg), Extract (3.32 g) and mordant (1.0 g)
3. Constituents of Aqueous Stain (20 ml): Distilled water (20 ml), thymol (10 mg), extract (3.32 g) and mordant (1.0 g).

Result

The study evaluated the staining capacity of dye extracted from *B. vulgaris* on the liver, kidney and testes using different grades of extracting solvents (water and 50% ethanol and 80% ethanol), pH (5.0 and 11.0). Photomicrographs of the stained sections were documented accordingly (figure 1-7).

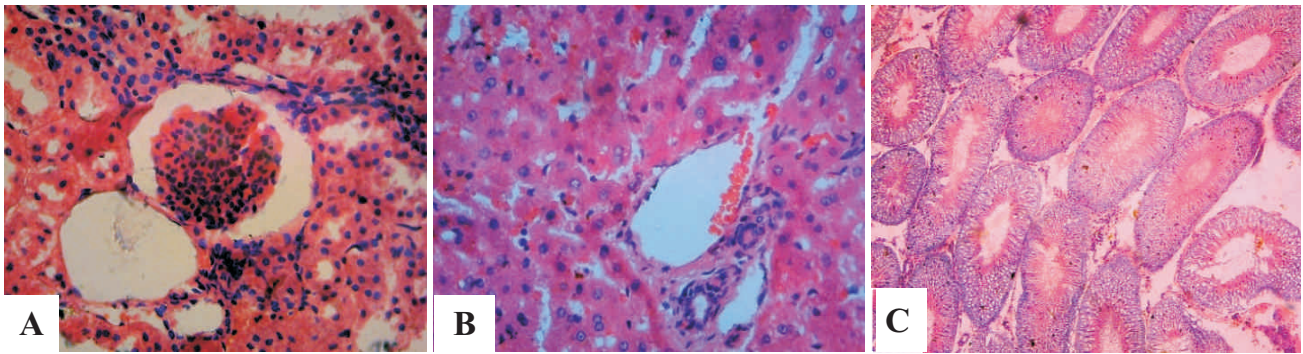


Figure 1: A. Photomicrograph showing well differentiated nucleus and cytoplasm of the Kidney (A), Liver (B) and Testis (C). Conventional Ehrlich Haematoxylin and Eosin Staining Technique (X400).

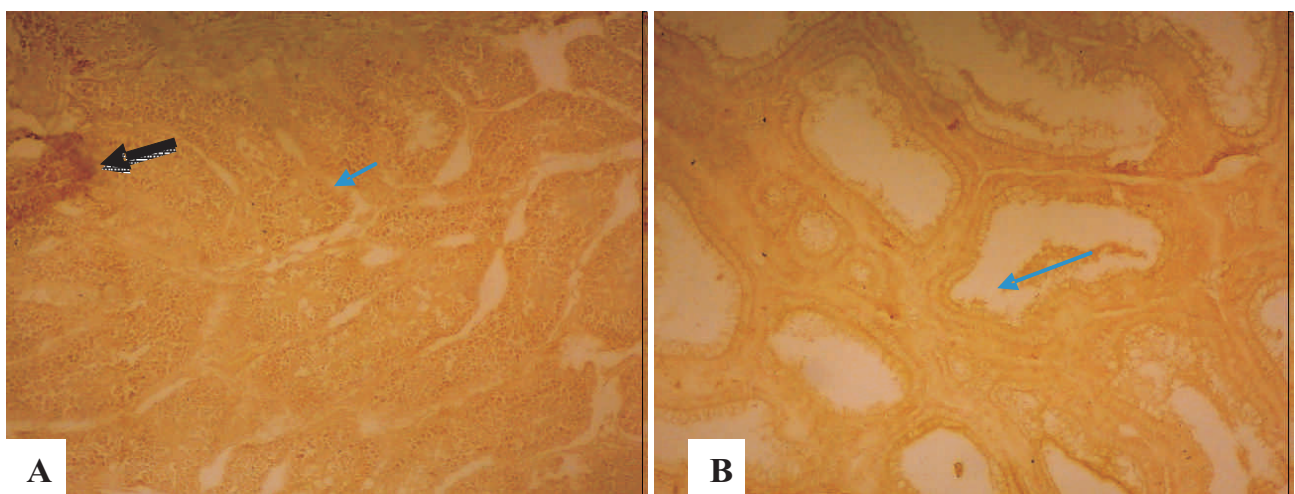


Figure 2 (A&B): Photomicrograph of sections from the testes stained with 50% *B. vulgaris* at pH 5.0. Tissue architecture stained in shades of yellow (blue arrow) to brown (black arrow) X100.

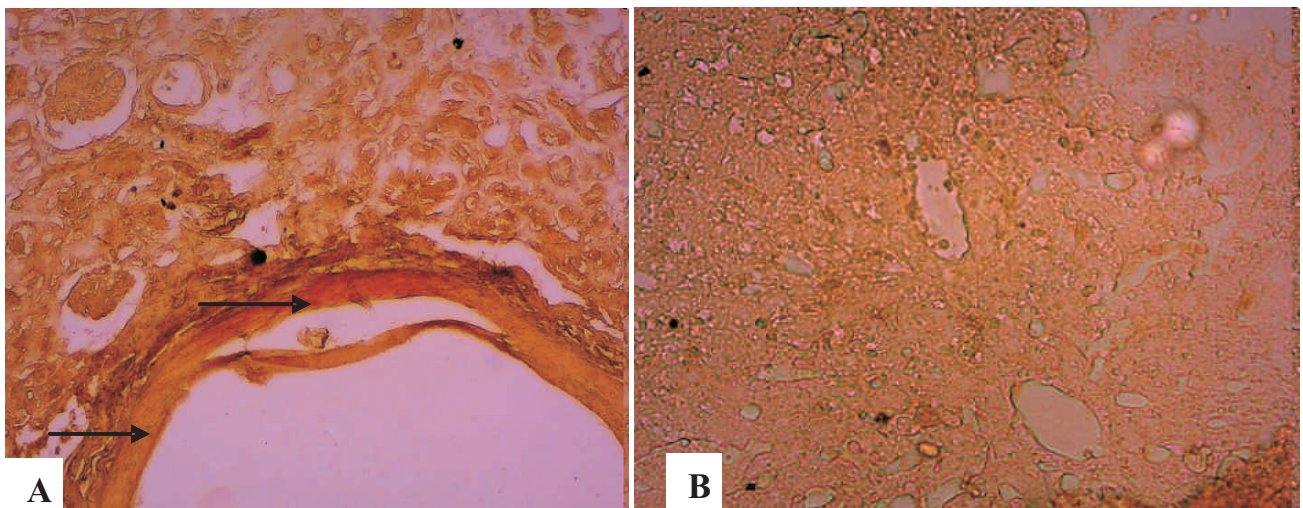


Figure 3: A. Photomicrograph of a kidney section stained with 80% *B. vulgaris* at pH 5.0. Section shows stained connective tissues in shades of yellow to brown (marked by black arrow); B. Photomicrograph of liver section stained with 80% *B. vulgaris* at pH 5.0 X100.

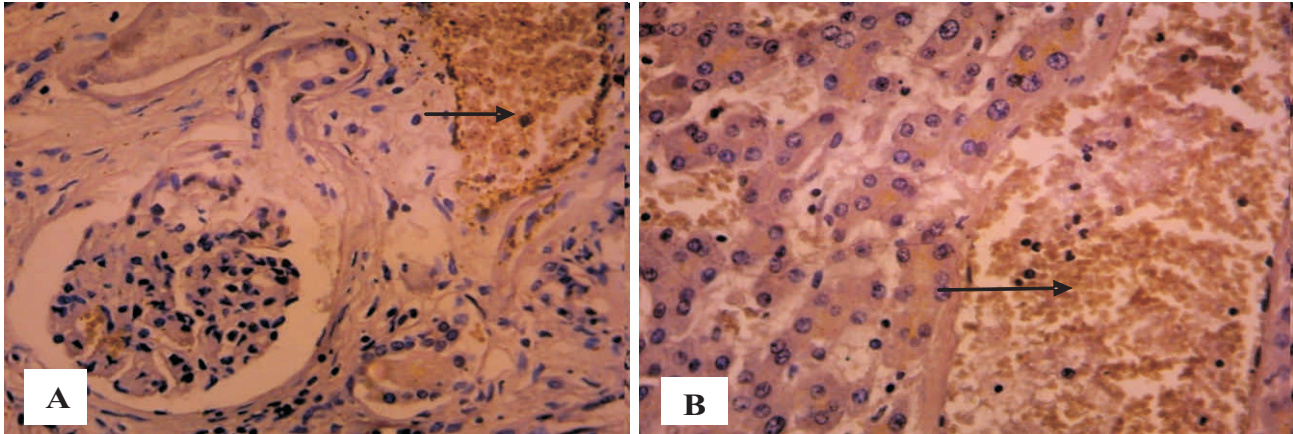


Figure 4: *Photomicrograph showing well differentiated nucleus and cytoplasm of Kidney and red blood cells (black arrow; brown). Demonstrated by Harris Haematoxylin and 50% B. vulgaris dye at pH 5.0 (X400). B. Photomicrograph showing well differentiated nucleus and cytoplasm of hepatic cell in the liver. Demonstrated by Harris Haematoxylin and 50% B. vulgaris dye at pH 5.0 (X400).*

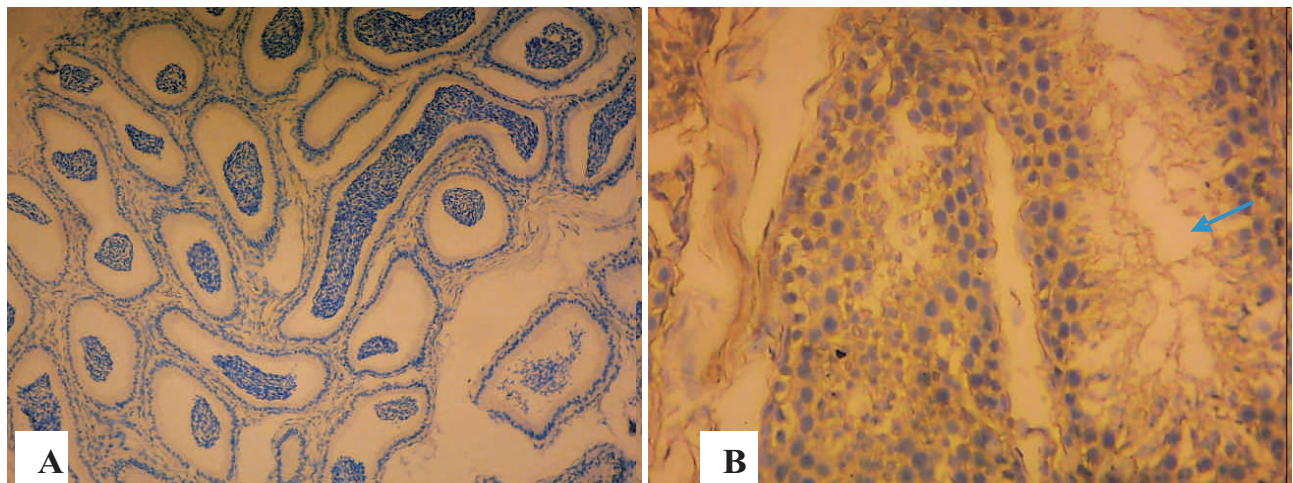


Figure 5: *A. Photomicrograph of the testis section stained with Harris haematoxylin and 50% B. vulgaris at pH 5.0. as the counter stain (X100); B. Photomicrograph of the testis section stained with Harris haematoxylin and 50% B. vulgaris at pH 5.0 as the counter stain (X400). Blue arrow tales of spermatozoa (brownish in colour).*

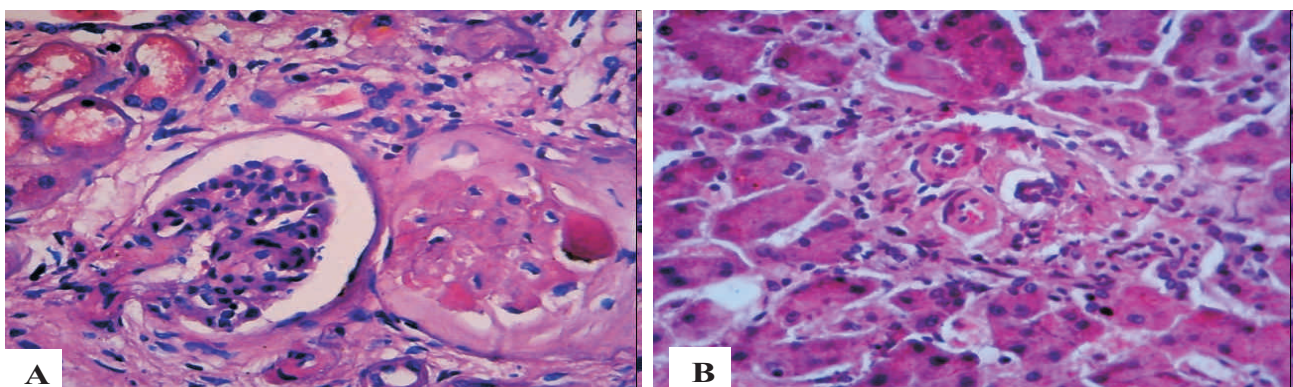


Figure 6: *A. Photomicrograph showing well differentiated glomerulus in the Kidney. Demonstrated by Harris Haematoxylin and equal parts of 50% B. vulgaris dye (pH 5.0) and 1% Eosin (X400); Figure 8:* *A. Photomicrograph showing well differentiated glomerulus in the Kidney. Demonstrated by Harris Haematoxylin and Equal parts of 80% B. vulgaris dye (pH 5.0) and 1% Eosin (X400).*

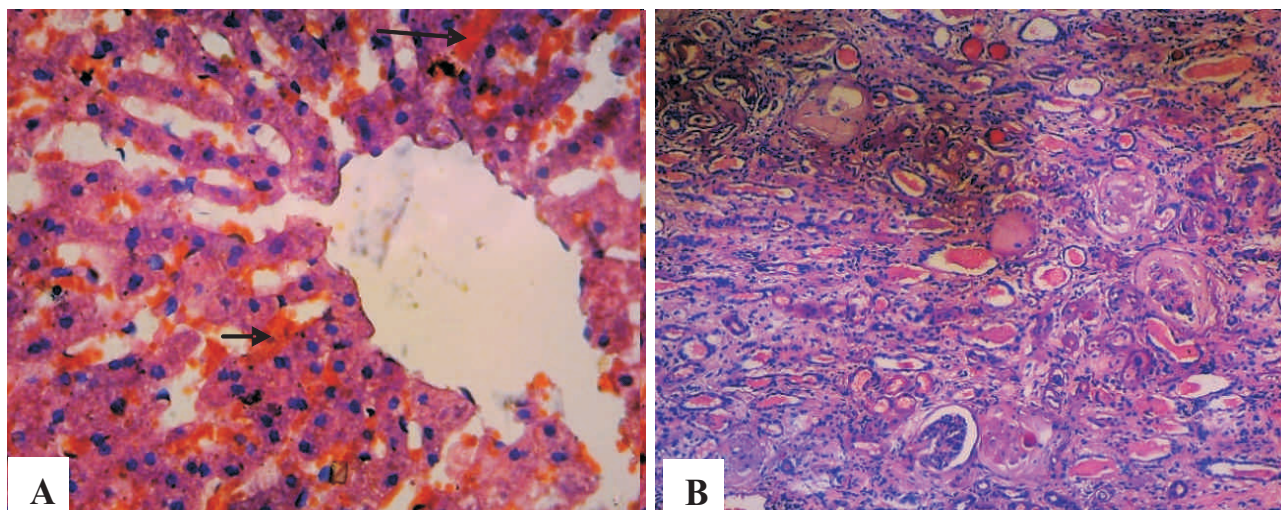


Figure 7: A. Photomicrograph showing the hepatocytes and red blood cells (black arrows) of the liver. Demonstrated by Harris Haematoxylin and equal parts Aqueous *B. vulgaris* dye (pH 5.0) and 1% Eosin (X400). B. Photomicrograph showing well differentiated glomerulus in the Kidney. Demonstrated by Harris Haematoxylin and equal parts 50% *B. vulgaris* dye extract (pH 5.0) and 1% Eosin (X100).

Discussion

Beta vulgaris has been used over the years for different purposes such as natural food colorant (Roy *et al.*, 2004; Koul *et al.*, 2002). It has also been used for demonstrating internal structures of parasites in shades of red and yellow (Okolie, 2006). Following the latter successes, this study evaluated staining potential of *B. vulgaris* on tissues. The good cytoplasmic staining offered by 50% ethanol may be due to high solubility index of alcohol and water. Although, tissues stained in *B. vulgaris* were not superior to tissues stained by the conventional H&E technique, the findings of this study suggest that the combination of the former and Eosin may offer superior staining quality to the conventional H&E technique. The staining results obtained from 50% ethanol extract of *B. vulgaris* and the combination of 50% ethanol extract of *B. vulgaris* and Eosin are similar to the reports of Al-Amura *et al.* (2012) and Cheng *et al.* (2014). Using 80% ethanol extracted dye, some connective tissues were also demonstrated. Considering pH, dye extracted at pH 5.0 successfully demonstrated the cytoplasm. This suggests that the staining components of *B. vulgaris* may only be found in acidic spectrum. Alum and iron mordants offered no additional advantage over sections stained without mordant

since the dyes did not successfully stain the nuclei. In conclusion, this study suggests that ethanol extract of *B. vulgaris* could be used as a cytoplasmic dye. However, more studies should be carried out on the plant so as to extract dyes of other colours for possible nuclear demonstration.

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