

## Original Research Article

# Antidiuretic Activity of the Methanol Extract of *Aporusa lindleyana* Wight (Euphorbiaceae) Baillon in Rats

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### Abstract

**Purpose:** To determine the diuretic activity of *Aporusa lindleyana* in rats following its claimed use in Sri Lankan traditional medicine.

**Methods:** Three doses (500, 1000 and 1500 mg/kg) of the methanol extract (ME) of *Aporusa lindleyana* (Leaf and bark in 5:1 ratio) were orally administered to female, adult Sprague Dawley rats. Furosemide, vasopressin (antidiuretic hormone) and distilled water were used as diuretic reference, antidiuretic reference and control, respectively. Urine output was recorded up to 6 h at hourly intervals. To investigate the mode of action, pH, specific gravity, conductivity, total dissolve solids, Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, leucocytes, nitrite, urobilinogen, protein, blood, ketones, bilirubin and glucose were tested in the urine of 500 mg/kg treated and control rats. Six hours later, serum electrolyte levels (Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup>) were evaluated. Glomerular filtration rate (GFR) was also determined in terms of creatinine clearance. Overt toxicity, hepatotoxicity, nephrotoxicity and a phytochemical studies were conducted.

**Results:** Significant ( $p < 0.05$ ) reduction of urine output (by 45 %) and aldosterone secretion index (Na<sup>+</sup>/K<sup>+</sup>) were observed while sodium (by 38 %) and potassium (by 114 %) levels were increased significantly ( $p < 0.05$ ). GFR was not changed significantly ( $p > 0.05$ ). Further, the methanol extract (leaf/bark in 5:1 ratio) was non-toxic in terms of overt signs of toxicity, serum alanine transaminase (ALT) and aspartate aminotransferase (AST), urea and creatinine levels. Phytochemical evaluation revealed the presence of alkaloids, unsaturated sterols, unsaturated terpenes, unsaturated lactones, lucocyanins, tannins and polyphenols and cyanogenic glycosides.

**Conclusion:** The methanol extract of *Aporusa lindleyana* has moderate and safe oral antidiuretic activity.

**Keywords:** *Aporusa lindleyana*, Antidiuretic, Diuretic, Toxicity, Phytochemicals, Sri Lankan traditional medicine.

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## INTRODUCTION

Sri Lanka has an enormous diversity of medicinal plants. Therapeutic value or biodynamic activities of many medicinal plants have still not been proven scientifically. *Aporusa lindleyana* (Wight) Bali (Family: Euphorbiaceae), (Kebella or Barawa-

Embilla in Sinhala and Vittil, Kodali, Vettikan or Vitti in Tamil) is a medicinal plant, distributed in Sri Lanka and South India [1,2]. In Sri Lanka, it is preferably grown in intermediate zone areas and secondary forest and river banks. It is an evergreen tree up to 18 m tall, having branched brown coloured smooth stem. Leaves are

deciduous, ovate-lanceolate in shape with undulate margins; leaf tip is acute and rounded to obtuse at the base [1,2]. In Sri Lankan traditional medicine, diuretic activity of *A. lindleyana* has been claimed as a main medicinal activity [1]. But its diuretic activity has not been scientifically proven. Other medicinal activities of this plant have been reported to possess haemostasis, reddened/watery eyes, detoxifier for glory lily poisoning and as a blood purifying drug [1,3]. Also this plant has been used for several folk medicinal recipes in treatment of eye diseases, loose motions, cataract, diabetes, all types of anaemia in man and constipation in cows [1,3].

Scientific studies have proven that pet ether, chloroform, methanol and water extracts of this plant has moderate to very good antibacterial activity (against *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumonia*), compared with the standard drug tetracycline as well as antifungi activity (against *Penicillium chrysozenous*, *Candida albicans*, *Aspergillus niger* and *Trichoderma vridar*), compared with the standard drug fluconazole [4]. All the foregoing extracts of the bark have moderate analgesic activity on Swiss albino male mice [4]. The aqueous and alcoholic extracts of *A. lindleyana* possess anti-diabetic activity on alloxan-induced diabetic rats [5]. Ethanolic extract of the root of the plant has shown antiviral activities by *in vitro* HBsAg binding, HBV-DNA polymerase inhibition, RT (non-isotopic) inhibition and HSV inhibition [6]. Decoction of *A. lindleyana* roots is rich in antioxidants, phenolics and flavonoids. GC-MS studies also has proven that it has many phytochemicals such as squalene, phthalic acid and 1,2 benzene dicarboxylic acid, butyl cyclohexyl ester, etc [7]. The *in vitro* cytotoxicity studies against normal vero cell lines indicate non-toxic nature of ethanolic of extract of *A. lindleyana* root [7].

The aim of this study was to investigate the validity among Sri Lankan traditional practitioners that *A. lindleyana* has diuretic potential using the methanol extract. Methanol extract of plants are widely used and effective in determination of biodynamic activities such as diuretic/antidiuretic activities and phytochemical evaluations [9,10].

## EXPERIMENTAL

Plant materials were collected from Pinnaduwa Medicinal Plant Garden (6° 4' 13" N, 80° 16' 1.21" E, elevation about 84 meters), Department Ayurveda, during September – November, 2011

at day time (between 10.00- 13.00 h). The plant materials were identified and authenticated by Dr. HS Kathriarachchi at Department of Plant Science, University of Colombo. A voucher specimen (no. SKG/EU/001) was deposited at the museum of the Department of Botany, University of Colombo.

### Preparation of extract

Plant materials were washed using running tap water. Then, plant parts were kept, under shade at room temperature (30 - 32 °C) until a constant weight (about 14 – 21 days) was obtained. Leaves and bark of shade-dried plant materials were crushed separately, using mechanical grinder (Blender FMI, Waring, New Jersey, USA). Grounded plant materials were homogenized in size using sieving. The plant materials (900 g), leaves and bark (5:1 ratio) were defatted by maceration process with petroleum ether (6 L) for 7 days with occasional shaking. Defatted plant materials were extracted into methanol by refluxing on a water bath (Buchi Water bath, B-481, Buchi Labortechnik, Switzerland) at 65 °C for 18 h (300 g on each occasion with 2 L for 6 h x 3 times).

Refluxed methanol portion was concentrated using rotary evaporator (EYELA, SB-650, Tokyo Rikakikai Co. Ltd, Japan) at 40 °C. Final semisolid residue was freeze dried using freeze-drying machine (Labconco, Labconco Corporation, USA) at -58 °C to obtain a completely dry solid (79.98 g, 8.89 %) and weighed (Libror, EB-3200D, Shimadzu Corporation, Japan). The crude, methanol extract (ME) was stored in airtight containers at -20 °C until it is used. Well formulated homogeneous water suspension was prepared with this crude extract daily, 5 - 10 min before use.

### Experimental animals

Healthy adult Sprague Dawley female rats (weight: 180 - 250 g) were used in the study. The animals were kept in plastic cages (six per cage) in an animal house under standard animal house conditions (temperature: 28 - 32 °C, photoperiod: approximately 12 h natural light per day, relative humidity: 50 - 55 %). The animals were free to access pelleted food (Master Feeds Ltd, Colombo, Sri Lanka) and clear drinking water. Except at the time of the experimental procedure, the animals were handled only during cage cleaning. All the experiments were conducted in accordance with internationally accepted laboratory animal use and care guidelines [8,11,12] and obtained clearance (EC/11/188) from Ethics Review Committee of Faculty of

Medicine, University of Colombo, for animal experimentation.

### Drugs and chemicals

Methanol (BDH Chemicals Ltd, Poole, England) was used as extracting solvent. Furosemide (State Pharmaceutical Manufacturing Company, Ratmalana, Sri Lanka) was used as diuretic reference and at the given dose of 13 mg/kg [11]. An antidiuretic hormone (vasopressin, Vasopin®, Samarth Pharama Pvt. Ltd, Mumbai, India) was used as antidiuretic reference at a dose of 0.13 ml/rat. All the other chemicals used for phytochemical analysis were purchased from BDH Chemicals Ltd, Poole, England.

### Evaluation of diuretic/antidiuretic activity

Forty nine rats were deprived of water for 18 h. The rats were then orally administered with 15 mL of saline (NaCl, 0.9 % w/v) to impose uniform water load [9]. After 45 min, urinary bladder of each rat was emptied by gentle compression of the pelvic area and by pulling of the tail. The rats were then randomly divided into six groups (assigned group numbers, I - VI) and treated either orally (Group-II–VI) or intraperitoneally (Group-I) according to the following manner, Group-I (n = 6) 0.13 ml/rat antidiuretic hormone (ADH), Group-II (n = 6) 13 mg/kg furosemide, Group-III (n = 6) 500 mg/kg ME, Group-IV (n = 5) 1000 mg/kg ME, Group-V (n = 5) 1500 mg/kg ME, Group-VI (n = 21) 2 mL distilled water according to accepted laboratory handling techniques for rats [8]. Urine output was measured hourly over 6 h from the point of administration of saline to the rats.

Diuretic/antidiuretic activity was determined as the ratio of urinary output of treated to control group; diuretic potency as ratio of urinary out of treated to reference group; urinary excretion as the ratio of total urinary output to total volume of liquid administered; saline excreted as the ratio of urine volume excreted to volume of saline loaded; and change in urine output as the ratio of increased/decreased urine volume to urinary output of control group. All the foregoing parameters were expressed as a percentage [11].

### Evaluation of mechanism of action

The collected urine for the 500 mg/kg dose was tested for leucosites, nitrite, urobilogen, protein, pH, blood, specific gravity, ketone, bilirubine and glucose using urine test kit (Cortez Diagnostics, Inc, USA). Electrolytes such as Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup> and Ca<sup>2+</sup> ions concentration were measured (in

ppm) using atomic absorption spectrophotometer (FAAS, GBC 935 plus, GBC Scientific Equipment Pty. Ltd, Victoria, Australia). Conductivity and total dissolve solids (TDS) were measured using conductivity meter (Jenway, 4510, Bibby Scientific Ltd, UK). The pH was measured using pH meter (Eutech, pH 510, Eutech Instruments, Singapore). Diuretic indices; sodium saluretic index (=Na<sup>+</sup> ion in treated group/Na<sup>+</sup> ion in control group), potassium saluretic index and aldosterone secretion index (Na<sup>+</sup>/K<sup>+</sup>) were calculated for 500 mg/kg dose of ME [11]. After 6 hrs administration of 500 mg/kg of ME, blood was withdrawn from tail and tested for selected electrolytes (Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup> and Ca<sup>2+</sup>) using atomic absorption spectrophotometer (FAAS, GBC 935 plus, GBC Scientific Equipment Pty. Ltd, Victoria, Australia) to check for any alteration of main electrolyte ions in serum.

### Estimation of creatinine clearance (C<sub>cr</sub>)

Ten (n=10) rats were deprived of water for 18 h and orally administered with 15 mL of saline (NaCl, 0.9 % w/v) to impose a uniform water load. After 45 min, urinary bladder of each rat was emptied by gentle compression of the pelvic area and by pulling of the tail. Then, rats were randomly divided into two groups (n=5 per group). First group was treated orally with 500 mg/kg of ME and other group was given distilled water (2 ml) orally. All rats were individually placed in metabolic cages. From the point of rat administration of saline, total urine output was measured, and thereafter, blood was drawn via the tail 24 h later [8]. Blood serum was separated and tested for creatinine using Randox creatinine kit (Randox Laboratories Ltd, UK) colourimetrically (UV –Visible Spectrophotometer, Jasco, V-560, Tokyo, Japan). Glomerular filtration rate (GFR) is measured in terms of C<sub>cr</sub> using creatinine in blood (P<sub>cr</sub>) and urine (U<sub>cr</sub>) and urine flow rate (V<sub>t</sub>),  $GFR = (U_{cr} \times V_t) / P_{cr}$  [13].

### Evaluation of acute and subchronic toxicity

Twelve rats (n = 12, weighing 190 - 250 g) were randomly divided into two groups (n=6 each). Distilled water (2 ml/rat) was orally given into group which served as the control group. Treated group were orally treated with the highest dose of the extract (1500 mg/kg) for 30 consecutive days. During this period, each rat for both the extract and the control group was observed for the following overt toxicity signs; salivation, lacrimation, breathing distress, ptosis, stupor, squint, teeth exposure, writhing, convulsions, tremors, yellowing of fur and loss of fur, stress (erection of fur and exophthalmia), behavioral abnormalities (biting and scratching, licking of

tail, paw) and diarrhoea. This observation was done daily [9,11]. On the 31st day (one day post-treatment) rats from all the groups were anaesthetized with diethyl ether and blood was withdrawn from the tail under aseptic conditions [8,11]. Blood samples were kept at room temperature (30 °C) for 1 h, stored at 4 °C overnight and centrifuged at 4000 rpm for 20 min. Serum layer was separated and tested for alanine transaminase (ALT), aspartate transaminase (AST), urea and creatinine levels colourimetrically (UV-Visible Spectrophotometer, Jasco, V-560, Tokyo, Japan) using respective Randox test kits (Randox Laboratories Ltd, London, UK) as per instructions given.

### Phytochemical screening

The methanol extract of *A. lindleyana* plant (leaves and bark in 5:1 ratio) was subjected to qualitative determination of alkaloid, flavonoids, phenols, coumarines steroids, saponins, tannins, amino acids and peptides as described by Fong's phytochemical methods [14].

### Statistical analysis

The data are expressed as mean  $\pm$  SEM. Statistical analysis was performed using Mann-Whitney U-test by Minitab 14.1 computer package. Significant level was set at  $p < 0.05$ . Linear regression analysis was performed to assess dose dependencies.

## RESULTS

Urine volumes in the rats administered with 500 mg/kg of ME dose and ADH (0.13/rat) decreased significantly ( $p = 0.0318$  and  $0.0047$ , respectively). However, there was no significant ( $p > 0.05$ ) difference of urine output of 1000 mg/kg and 1500 mg/kg doses compared to the control group ( $p = 0.1184$ ,  $0.6488$  respectively) (see Table 1). The urine output decreased with the decreasing doses of ME. The antidiuretic activity of ME was inversely dose-dependent ( $r^2 = -98.6$ ).

**Table 1:** Effect of oral administration of methanol extract (ME) of *Aporusa lindleyana* on urine output of rats

Treatment	Total urine output (ml)	Change of urine volume (%)	Diuretic/antidiuretic		% Saline excreted	% Urinary excretion
			Action	Potency		
Furosemide, 13 mg/kg	1.98 $\pm$ 0.20*	Ic 57%	1.57	1.00	13.22 $\pm$ 1.35*	11.66 $\pm$ 1.19*
ME, 500 mg/kg	0.70 $\pm$ 0.08*	Dc 45%	1.80	0.71	4.66 $\pm$ 0.54*	4.11 $\pm$ 0.48*
ME, 1000 mg/kg	0.87 $\pm$ 0.08	Dc 31%	1.44	0.57	5.84 $\pm$ 0.49	5.15 $\pm$ 0.44
ME, 1500 mg/kg	1.09 $\pm$ 0.15	Dc 11%	1.12	0.44	7.48 $\pm$ 1.00	6.60 $\pm$ 0.88
ADH, 0.13 ml/rat,	0.50 $\pm$ 0.03*	Dc 60%	2.53	1.00	3.33 $\pm$ 0.23*	2.93 $\pm$ 0.20*
DW, 2 mL	1.26 $\pm$ 0.12	-	-	-	8.41 $\pm$ 0.78	7.42 $\pm$ 0.69

\* $p < 0.05$ , compared to control by non-parametric test (Mann Whitney U-test);  $n = 5, 6$  (or 21 for the control group that received distilled water, DW); % change in urine volume was done in comparison with the control group; Ic = increase; Dc = decrease

**Table 2:** Effect of oral administration of the methanol extract (ME) of *Aporusa lindleyana* (500 mg/kg) on some urine parameters (up to 6 h) on rats

Parameter	Control group	500 mg/kg extract
pH	6.41 $\pm$ 0.29	6.53 $\pm$ 0.04
Specific Gravity (SG)	1.025 $\pm$ 0.0016	1.026 $\pm$ 0.0015
Conductivity / $\mu$ S	17.72 $\pm$ 2.37	25.79 $\pm$ 3.76
Total Dissolve Solids (TDS) /mgL <sup>-1</sup>	9.75 $\pm$ 1.76	12.86 $\pm$ 1.02
Na <sup>+</sup> (ppm)	5346.67 $\pm$ 564.19	7373.33 $\pm$ 612.07*
K <sup>+</sup> (ppm)	2614.00 $\pm$ 321.30	5581.50 $\pm$ 697.13*
Ca <sup>2+</sup> (ppm)	387.20 $\pm$ 30.58	216.66 $\pm$ 88.32
Mg <sup>2+</sup> (ppm)	631.00 $\pm$ 78.95	791.67 $\pm$ 75.82
Sodium Saliuretic Index	1.0	1.38
Potassium Saliuretic Index	1.0	2.14
Aldesterone Secretion Index (Na <sup>+</sup> /K <sup>+</sup> )	2.11 $\pm$ 0.19	1.40 $\pm$ 0.14*
Leucosites (cells/ $\mu$ L)	N.D	N.D
Nitrite (in terms of organisms/ml)	N.D	N.D
Urobilogen ( $\mu$ mol/L)	N.D	N.D
Protein (mg/dL)	N.D	N.D
Blood (cells/ $\mu$ L)	N.D	N.D
Ketone (mg/dL)	N.D	N.D
Bilirubine (+/-)	N.D	N.D
Glucose (mg/dL)	N.D	N.D

\* $p < 0.05$ , compared to control by nonparametric test (Mann Whitney U-test); N.D = not detected

As shown in Table 2, 500 mg/kg of ME did not significantly ( $p < 0.05$ ) alter the pH, SG, conductivity and TDS compared to the control group ( $p = 0.1282, 0.8312, 0.0927$  and  $0.2298$  respectively). On the other hand, sodium (by 38 %) and potassium (by 114 %) levels significantly ( $p < 0.05$ ) increased compared to control group ( $p = 0.0453, 0.0131$  and respectively) and Na<sup>+</sup>/K<sup>+</sup> ratio was significantly ( $p < 0.05$ ) decreased ( $p = 0.0131$ ) compared to control group (see Table 2). Any abnormality of urine (in terms of leucosites, nitrite, urobilogen, protein, blood, ketone, bilirubine and glucose) was not detected.

Further, significant alteration ( $p > 0.05$ ) of creatinine clearance was not observed in test group ( $4.61 \pm 1.62$  ml/h) compared to control group ( $6.83 \pm 2.15$  ml/h) ( $p = 0.5309$ ).

**Table 3:** The effect of 30 days oral administration of methanolic extract (ME) of *Aporusa lindleyana* (1500 mg/kg) on some serum parameters on rats Mean $\pm$ SEM)

Parameter	Test Group	Control Group
Urea (mg/dL)	33.60 $\pm$ 2.981	34.82 $\pm$ 2.398
Creatinine (mg/dL)	1.20 $\pm$ 0.067	0.99 $\pm$ 0.141
ALT (U/l)	69.84 $\pm$ 7.557	59.36 $\pm$ 14.968
AST (U/l)	127.75 $\pm$ 22.055	139.10 $\pm$ 69.173

In the toxicity study, 1500 mg/kg dose of ME did not provoke any overt signs of toxicity, stress, behavioural abnormalities, aversive behaviour or diarrhoea. In addition, none of the treated rats died. After feeding for 30 consecutive days (see Table 3), none of investigated serum parameters (urea, creatinine, ALT and AST levels) were significantly ( $p < 0.05$ ) altered ( $p = 1.0000, 0.4386, 0.6985$  and  $0.5186$  respectively).

### Phytochemical profile of extract

Alkaloid test showed presence of primary, secondary or tertiary alkaloids but absence of quaternary alkaloids or amine oxides. Lieberman-Burchard test and the Salkowski test indicated the presence of unsaturated sterols and triterpenes. Unsaturated lactones and 2-deoxy sugar test gave positive results. Flavonoids and leucoanthocyanin screening results showed absence of cyanidin but presence of leucocyanins and flavonoids. Borntrager test and modified Borntrager test gave negative results due to the absence of anthraquinones. A positive result for picrate paper test indicates the presence of cyanogenic glycosides. Froth test gave negative results for saponins.

## DISCUSSION

This study examined the effect of methanolic extract of *A. lindleyana* (ME) on urine output of rats. The results showed that ME has moderate antidiuretic activity (in terms of antidiuretic action, antidiuretic activity, percentage of urinary excretion, percentage saline excreted, percentage change of urine output and aldosterone secretion index compared to control group). This does not support the claim made by Sri Lankan traditional medicine that it is a potent oral diuretic. It is an important finding with therapeutic implications because about 35 % of Sri Lankan population still mainly depends on Ayurvedic and Sri Lankan traditional medicines for primary healthcare [15].

The lowest dose of the extract (500 mg/kg) showed the highest antidiuretic activity while highest dose demonstrated the lowest antidiuretic activity, thus indicating an inverse dose-relationship. It significantly decreased urine output (by 45 %) compared to control group. The urine of rat treated with the lowest dose of ME was markedly hypernatremic (in terms of urinary Na<sup>+</sup> level and sodium saliuretic index) and hyperkalemic (in terms of urinary K<sup>+</sup> level and potassium saliuretic index). Urinary sodium and potassium levels were significantly increased. Aldosterone secretion index (Na<sup>+</sup>/K<sup>+</sup>) decreased significantly. GFR (in terms of creatinine clearance) indicated that ME did not alter ultrafiltration process and clearly the dose not act on glomerulus of nephron.

The antidiuretic action of a herbal drug may be mediated by several methods [15,16]. Some antidiuretics impair GFR [17]. The ME of *A. lindleyana* did not decrease the GFR of treated rats. Therefore, antidiuretic activity of ME is unlikely to be mediated via this mechanism. An antidiuretic action can be mediated by increasing the ADH secretion from posterior pituitary and/or by having agonist activity to the ADH V2 receptor present in the distal convoluted tubule and collecting duct of nephrons, which would enhance water reabsorption possibly increasing urinary sodium and potassium level as evident in this study [13].

Antidiuretic effects in the human body are brought about by ADH and aldosterone [13]. Both hormones help to retain water but the mechanism by which it reabsorbs the water is different. It is claimed that ADH has the ability to reabsorb urea and it helps to reabsorb water along with it. In contrast, aldosterone reabsorbs

sodium and this helps in the reabsorption of water. As such, these two modes of action may be possible for the antidiuretic action of ME of *A. lindleyana*. Alternatively, increased secretion of aldosterone from adrenal cortex and/or agonist activity to aldosterone can induce antidiuresis. Such a mode of action is also possible since there was a significant reduction in aldosterone index ( $\text{Na}^+/\text{K}^+$  ratio) and significant increase in urinary potassium level. Thus, the antidiuretic action of ME is likely to be mediated by ADH and aldosterone mechanisms.

It is reported that the alkaloids, dioscorine and dioscine, can produce antidiuretic effect [18]. Some steroid-derived alkaloids found in plants (veratridine) can inhibit water diuresis in unanaesthetized rats [19]. Among these secondary metabolites, it has been reported that some flavonoids (namely chrysin, oroxylin-A, baicalein, biochanin-A, and ellagic acid) possess the antiinflammatory, antidiuretic, anti arthritic, antimicrobial activities [20]. It is known that some plant (*Rubia tinctorium* L) anthraquinone derivatives have been used as anti-diuretic drugs [21]. One or few of these compounds can be present in the methanol extract of *A. lindleyana* plant (ME). Combination of action of this plant secondary metabolites may have produced this antidiuretic effect.

Toxicity studies showed that ME (up to 1500 mg/kg) is well tolerated (in terms of overt signs of toxicity), and did not produce any renotoxicity (in terms of serum urea, creatinine, leucocytes, nitrite, urobilogen, protein, pH, blood, specific gravity, ketone, bilirubine and glucose) and hepatotoxicity (in terms of serum ALT and AST). Furthermore, no mortality was evident during the toxicity studies. These observations suggest that ME of *A. lindleyana* may be safe for regular consumption.

## CONCLUSION

The methanol extract of *A. lindleyana* has moderate and safe oral antidiuretic activity. This may be useful in the management of polyuria.

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