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ResearchArticle

Anti-Diabetic and Bronchodilator Activities of *Pothos scandens* Linn Leaves

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ABSTRACT

Pothos scandens Linn, commonly known as Hatilata, belonging to the family Araceae, is a highly apparent plant in ayurvedic system of medicine for the treatment of various ailments. Since from long time the plant, *Pothos scandens* Linn. is known for its beneficial effects, fruits of *A. reticulata* were screened for phytochemicals and anti diabetic and bronchodilator activity. The shade dried fruits were extracted with methanol. Extracts were screened for the presence of phytochemical constituents like alkaloids, tannins, flavanoids, saponins and others. The results showed the rich presence of majority of phytochemical constituents which can be correlated with the possible significant medical potential. The *in-vitro* anti-diabetic study was run by α-amylase enzyme inhibition technique, the extract showed IC_{50} value (1.49±0.190 mg/mL) whereas standard acarbose showed (1.30±0.015 mg/mL). In OGTT method the methanol extract of the whole plant of *P. scandens*100 mg dose shows (*In-vivo*) slight hypoglycemic activity. 200 and 400 mg dose shows moderate hypoglycemic activity comparing with positive control (Gliclazide). The bronchodilator activity of selected plant extract in applying on Wister rat by counting the PCT (Pre convulsive time) shows % of protection 41.56% at 100mg/kg dose with comparison of Salbutamol. Hence, the present study focused leaves extract of the plant possessed source of bioactivity against the diabetic and asthma as well as justifying the use of this plant to treat many ailments in folk and herbal medicines.

Keywords: *Pothos scandens*, Phytochemical screening, Anti-diabetic, Bronchodilator activity, Acarbose, Salbutamol

INTRODUCTION

According to the World Health Organization (WHO), 80% of the world's population depends on traditional medicines for their primary healthcare, it suggested in improving the technologies for cultivation of medicinal plants [1]. Plants have been used frequently to treat common diseases in rural areas as western medicines are either too expensive or not available [2]. About 25% of prescribed drugs in the world originate from plants [3]. Drugs derived from unmodified natural products or drugs semi synthetically obtained from natural sources corresponded to 78% of the new drugs approved by the FDA between 1983 and 1994 [4]. The demand for more and more drugs from plant sources is increasing. Hence, there is need to screen medicinal plants for promising biological activities in order to discover novel drug candidates [5]. As Bangladesh has numerous plants, proper scientific evaluations are required to explore the potential of these plants for treating various diseases [6, 7]. *Pothos scandens* Linn is a climbing shrub having adventitious acrid roots. The leaves are obovate, lanceolate and coriaceous having a bright green colour. The plant is given for hysteria and snake-bite in Khagrachari, Bangladesh [8]. Sri Lankan tribal people use leaves of *P. scandens* to reduce swelling speedily in trauma area [9]. In China the plants are used as blood coagulant, wounds, tumors and drinking for anti-cough [10]. In India, the infusion of the leaves of this plant as a bath, is used for curing convulsions and epilepsy. The antipyretic studies on methanol extract of root showed significant reduction of temperature in pyrexia induced rats at 200 and 400 mg/kg doses [11]. Apart from that, the stem is also reportedly used to treat asthma, after being cut with camphor and smoked like tobacco [12]. Since this plant has important medicinal properties, the study has been undertaken as part of our research investigation on the crude methanol extracts of leaves of *Pothos scandens* were studied for anti-diabetic and bronchodilator activities and we, here in, report the results of our preliminary investigations for the first time.

MATERIALS AND METHODS

Collection of plant materials

The fresh leaves of *Pothos scandens* were collected from Chittagong Hill Tracts at 16th April, 2014 by using hand labor with appropriate manner. Collected parts were identified by using standard taxonomical methods, supervised by experts from Department of Botany, University of Chittagong, Bangladesh. Voucher specimens (Accession No. 9822 CTGUH) have been maintained in Chittagong University Herbarium for future reference.

Extract preparation

The collected plant parts were dried for two weeks under open air drying by natural heat with shading and pulverized into a coarse powder using a suitable grinder.

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The powder was stored in an airtight container and kept in a cool, dark, and dry place until further analysis. Approximately 600 g powder were then macerated separately as 150 gm in each of the 900ml 95% pure methanol and macerated for 7 days with 6 hours interval shaking[13]. Extracts were collected and filtered. The solvent was evaporated and concentrated extract was obtained. The extract was stored in well closed container[14, 15].

Preliminary phytochemical screening

Freshly prepared extract were subjected to standard phytochemical analysis to ensure the presence of major chemical constituents according to the described standard procedure [16, 17].

Animals

The anti-diabetic experiments were carried out using male Swiss albino mice (20-25 g each) and bronchodilator activity were carried out using Wister rat (200-250 g each) collected from Bangladesh Council of Science and Industrial Research (BCSIR), Chittagong laboratory. The animals had free access to food and water and they were housed under a natural (12 h each) light-dark cycle with access to standard pellet chow and water ad libitum. The animals were acclimatized for at least 5 days to the laboratory conditions before performing the experiments. The experimental protocol was approved by the Institutional Animal Ethics Committee USTC. In all experimental models, five animals were used in each group.

Anti-diabetic activity

In vitro α-amylase inhibitory activity

This study was performed by a modified starch iodine protocol [18]. In short, 1 mL of plant extract or standard of different concentration (0.1, 0.3, 0.5, 1.1, 1.3, and 1.5 mg/mL) was taken in pre-labeled test tubes. A volume of 20 µL of α-amylase was added to each test tube and incubated for 10 min at 37 °C. After the incubation 200 µL of 1% starch solution was added to each test tube and the mixture was re-incubated for 1 h at 37 °C. Then 200 µL of 1% iodine solution was added to each test tube and after that, 10 mL distilled water was added. Absorbance of the mixture was taken at 565 nm. Sample, substrate and α-amylase blank were undertaken under the same conditions. Each experiment was done in triplicate. IC_{50} value was calculated by using regression analysis.

$$\%\ \alpha\text{-amylase inhibition} = \left[1 - \frac{(SA - SBB) - SMB}{AAB}\right] \times 100$$

SA=Sample absorbance, SMB=Sample blank, SBB=Substrate blank, AAB=α-Amylase blank

In vivo Anti-diabetic activity

Twenty five experimental animals were randomly selected and divided into five groups denoted as group-I, group-II, group-III, group- IV and group V consisting of 5 mice in each group. Each group received a particular treatment i.e. control, standard (Gliclazide) and the dose of the methanol extract of *Pothos scandens* prior to any treatment, each mouse was weighed properly and the doses of the test samples and control materials were adjusted accordingly. The animals were individualized by marked as M-1=Mice 1, M-2=Mice 2, M- 3=Mice 3, M-4=Mice 4 and M-5=Mice 5. In order to administer the extract at doses of 100,200,400 mg/kg body weight of mice 150 mg of the extract were measured respectively and triturated in unidirectional way by adding of small amount of Tween-80 (a suspending agent). After proper mixing of extract and suspending agent, normal saline was slowly added. The final volume of the suspension was made up to 5.0 ml. To stabilize the suspension, it was stirred well by vortex mixture. For the preparation of standard (Glicazide) at the dose of 10-mg/kg body weight, 10 mg tablet was dissolved into 3.0 ml normal saline (0.9% NaCl). Glucose solution (10%) was collected from commercial market named 10% DA of Orion Pharmaceutical. Twenty five fasted mice were divided into five groups of five mice each. Each groups received treatment. By using glucometer blood glucose level is measured after 0, 30, 90 and 120 minutes of glucose loading, blood samples were collected from tail vein. After 30 min of extract administration all groups were treated with 10% glucose solution (2gm/kg body wt.)

In vivo Bronchodilator Activity

Apparatus

A rectangular Perspex glass, with a capacity of about 30 cubic inches. It has a door through which the animal is introduced and taken out of the chamber, air inlet and outlet tubes and a histamine inlet tube. The second component is a Wright nebulizer connected to an electric source. Closely attached to the nebulizer is a small bottle that contains freshly prepared 1% w/v betahistine phosphate (Sigma, UK) solution that is connected to the chamber by the histamine inlet tube. *Determination of Pre-convulsive Time (PCT)*

Table 1: Phytochmical screening of Methanolic Leaves Extract of *Pothos scandens* Linn

Phytochemicals	Methanolic Leaves Extract of *Pothos scandens* Linn
Reducing Sugar	+++
Alkaloids	++
Glycoside	++
Tannins	+
Flavonoids	+++
Steroids	+
Saponins	-

(+): Presence of chemical compounds, (-): Absence of chemical compounds

(+) < (++) < (+++): Based on the intensity of characteristics observation.

In this assay Wister rat were subjected to betahistine challenge and the time taken in seconds, to show the first signs of respiratory distress were observed by increased rippling spasm. This is known as the Pre-convulsive time (PCT). The apparatus for the determination of PCT consist of two main components. A rectangular Perspex glass, with a capacity of about 30 cubic inches. It has a door through which the animal is introduced and taken out of the chamber, air inlet and outlet tubes and a betahistine inlet tube as shown in figure .The second component is a Wright nebulizer connected to an electric source. Closely attached to the nebulizer is a small bottle that contains

Table 2: IC_{50} values (mg/mL) for *Pothos scandens* methanolic leave extract and acarbose in α-amylase inhibitory assay.

Extract/ Standard	Concentrations in mg/mL with (% Inhibition)						IC_{50} value (mg/mL)
	0.1	0.3	0.5	1.1	1.3	1.5	
Methanol extract	20±0.25	23.7±0.44	25±0.86	25±0.34	38±1.02	51±0.89	1.49±0.190[b]
Acarbose	34.4±0.67	37±0.93	36.9±0.34	40.25±0.88	49±1.21	49±1.01	1.30± 0.015[a]

Values are the mean of triplicate experiments and represented as mean ± SEM (n=3). Values in the same column with different superscripts are significantly different (P<0.05).

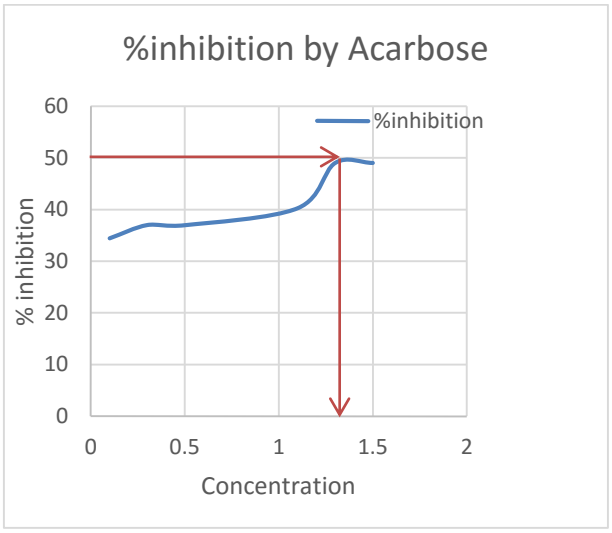

Figure 1: IC_{50} value (mg/mL) of Acarbose

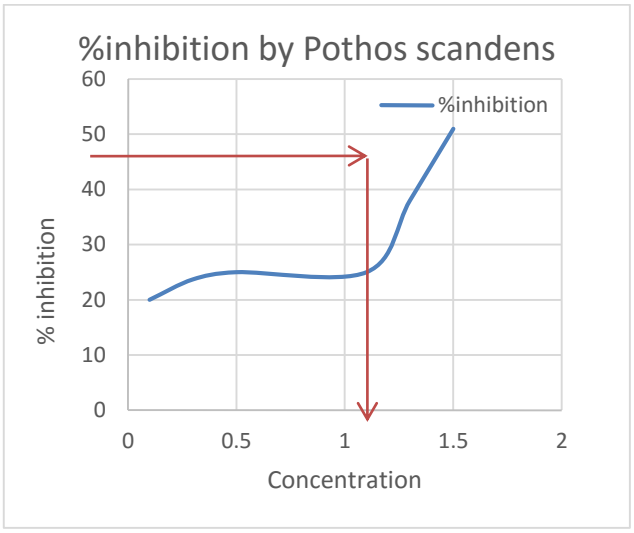

Figure 2: IC_{50} value (mg/mL) of Methanolic Leaves Extract of *Pothos scandens* Linn

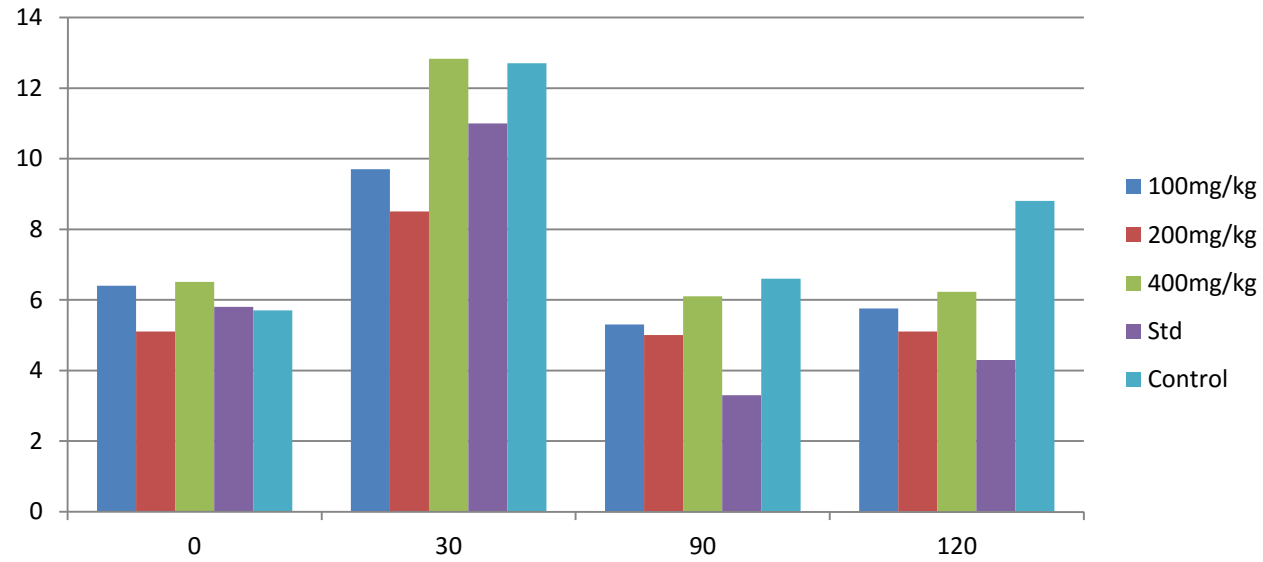

Figure 3: Glucose level of albino mice for 100mg/kg, 200mg/kg, 400mg/kg at certain time interval compare with std and controlled.

freshly prepared 1% w/v betahistine solution that is connected to the chamber by the betahistine inlet tube. On switching on the electric source, the nebulizer sprays the histamine into the glass chamber in the form of fine aerosol-like spray. The Perspex glass was well cleaned to ensure transparency and the door and the edges of the glass, greased with paraffin wax to ensure effective closing of the door. A stop clock was used to record the PCT. The experiments were carried out early in the morning when the animals had not eaten, to facilitate easy administration and effective absorption of the drugs. The fifteen wister rat were divided into three groups controlled, A and B. The five animals in each group were subjected to the betahistine challenge. The electric source was switched on for two minutes to saturate the chamber with the challenging betahistine solution (1% w/v). The animals were then individually introduced into the chamber, the door closed and the stop clock started simultaneously

while the spraying continued. Each animal was then observed for the first signs of respiratory distress by

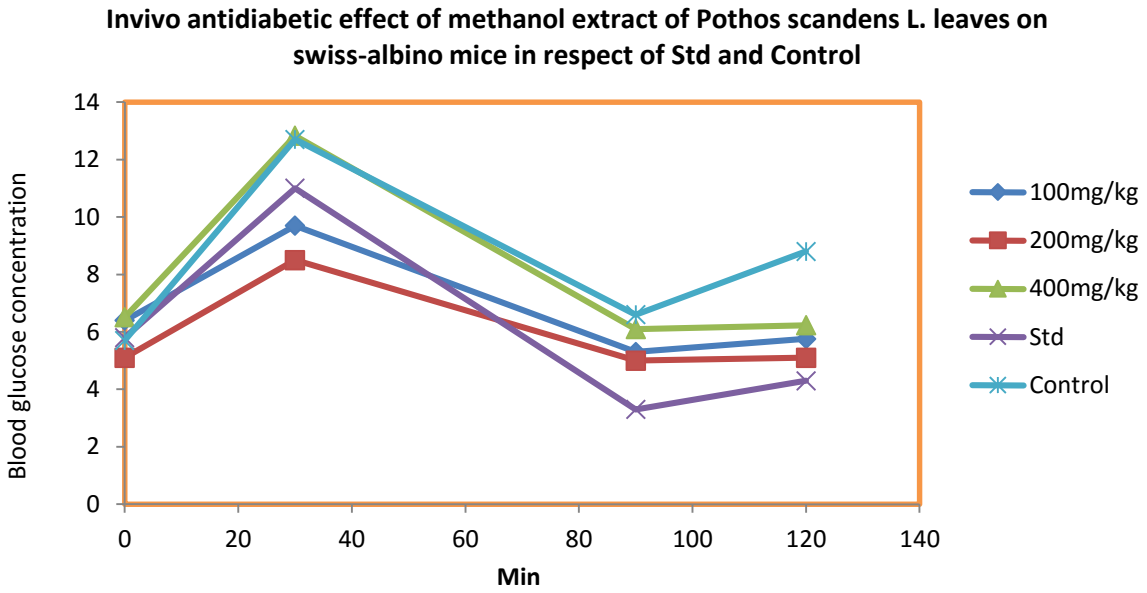

Figure 4: Comparative glucose level in mice for control, standard, 100mg, 200mg, 400mg concentrations of the sample

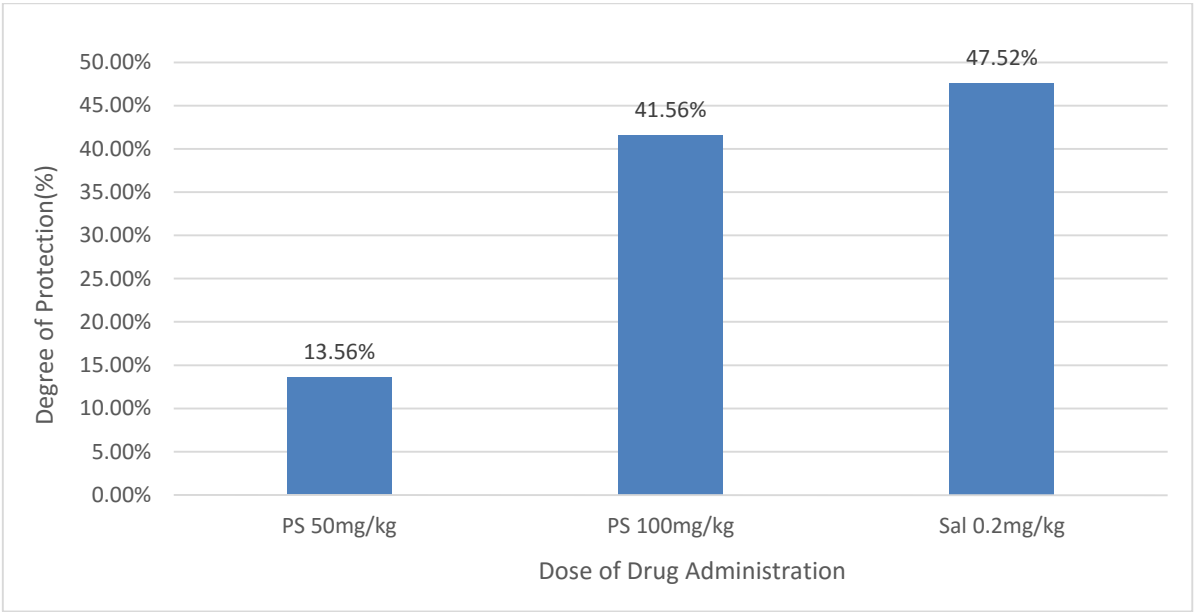

Figure 5: Comparative degree of protection in Wister rat for standard (Salbutamol 0.2mg/kg), 100mg/kg, 50mg/kgdose of the sample.

looking at the sides of the animal for increased rippling spasms or sometimes convulsive coughs [19]. The stop clock was stopped, the nebulizer switched off and the animal taken out of the chamber quickly. The animals were sometimes fanned and more so if any of them convulsed, to facilitate aeration and improved breathing. The period between the time each animal was introduced into the chamber and the time it showed the first signs of respiratory distress was noted and recorded as the PCT. This was repeated for all the animals in each group. The experiment was repeated on three occasions and in each case, a period of 3 days was allowed between the subsequent and previous experiments. In the first experiment designated as control, no drug was administered, only the challenging drug (betahistine 1% w/v) was used. In the second and third subsequent experiments, the PS and standard bronchodilator drug used as positive control were administered orally to the animals of different groups. Group A received 50 mg/kg of PS, group B 100 mg/kg of PS, the standard bronchodilator salbutamol at a concentration of 0.2 mg/kg body weight (which is the normal dose for humans). The drugs were administered 30 minutes before the PCTs were determined. The PCTs were used again to determine the degree of protection each drug gave to the animals as compared to the positive control of salbutamol [20].

Degree of protection of the Wister rat by the PS and salbutamol

Table 3: Glucose level of albino mice for 100mg/kg, 200mg/kg, 400mg/kg at certain time interval

Time(min)	0Min	Mean	30 min	Mean	90 min	Mean	120 min	Mean
Dose	(mmol/l)		(mmol/l)		(mmol/l)		(mmol/l)	
100mg/kg	5.3	6.4	6.4	9.7	5.2	5.53	4.9	5.76
	5.7		10.3		4.8		6.6	
	8.3		12.5		6.6		5.8	
	5.7	5.1	11.6	8.5	5.6	5.0	5.8	5.1
200mg/kg	3.6		5.1		4.4		3.9	
	6.2		8.9		5.1		5.5	
	8.83	6.51	17	12.83	6.8	6.1	7.2	6.23
400mg/kg	4.5		10		5.4		5.7	
	6.2		11.5		6.2		5.8	

Table 4: Determination of Pre-convulsive Time

Group A	PCT(T) sec	PCT(C) Sec	Degree of Protection	Avarage (%)
100mg /kg			(1-C/T) ×100%	
1	197		48.22	
2	148		48.69	
3	150		32	
4	165		32.18	41.56
5	172		40.69	
GroupB		102		
50mg/kg				
1	120		15	
2	130		21.53	
3	115		11.30	
4	109		6.42	13.56
5	118		13.56	

Table 5: Calculation of Degree of Protection

Control rat	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5
Pre-convulsive Time	108	99	102	106	95
Average			102		

Using the difference between the pre-convulsive time of the test drug (PS and salbutamol) and that of the control, the degrees of protection were calculated. As shown below by the formula Degree of Protection = (1-C/T) ×100% where C = PCT for the control and T = PCT for the test drug. The degree of protection offered by each of the test drugs were calculated using the above formula.

RESULTS AND DISCUSSION

The phytochemical screening (Table: 1) showed that methanolic extract of *Pothos scandens L.* leaf have abundance of flavonoids, alkaloids, glycoside like compounds. The *in-vitro* anti-diabetic study was run by α-amylase enzyme inhibition technique, the extract showed IC_{50} value (1.49±0.190 mg/mL) whereas standard acarbose showed (1.30±0.015 mg/mL) shown in figure: 1 and figure: 2. In figure 4: Comparative glucose level in mice for control, standard, 100mg, 200mg, 400mg concentrations of the sample and showed the dose dependent anti- diabetic activity. In the study of bronchodilator activity, Salbutamol drug used as a standard. Salbutamol is a well-known beta-2 receptor agonist with bronchodilator activity and is routinely used in the management of conditions of broncho constriction as occurs in asthma .The positive control drug, Salbutamol give the maximum protection of 47.52% whereas *Pothos scandens L.* leaf extract showed 41.56%. The results suggest that the *Pothos scandens L.* leaf extract have significant bronchodilator activity and justifying the traditional uses of the plant in the management of asthma.

CONCLUSION

The results of the present study show that the methanol extracts of *Pothos scandens L. leaf extrat* exhibit moderate anti-diabetic activities. The effect observed was dose-dependent and statistically significant at higher doses of treatment. Since the extracts have significant bronchodilator activity further investigations are required to understand the bronchodilator activity exhibited by methanol extract of *Pothos scandens L.* leaf

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