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EVALUATION OF ANTIEPILEPTIC ACTIVITY OF METHANOLIC FLOWER EXTRACT OF *ROSA DAMASCENA AND* METHANOLIC LEAF EXTRACT OF *PANDANUS FASCICULARIS* IN RATS

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ABSTRACT:

Epilepsy is a common chronic neurological condition. In the present study, a poly herbal extract comprising of flower of Rosa damascena, a medicinal plant used in many neurological disorders, convulsion and used as anti-HIV, antibacterial, antioxidant, antitussive, hypnotic, antidiabetic, and relaxant effect. The leaves of Pandanus fascicularis are thought to be useful in leprosy, smallpox, scabies and diseases of the heart and brain. Ayurvedic preparations along with two medicinal plants were evaluated for its protective effect against seizures induced by Maximal Electro shock (MES) method in rats. The present study is to evaluate the antiepileptic activity of methanolic flower extract of Rosa damascena and methanolic leaf extract of Pandanus fascicularis in rats. A daily dose of 250 and 500 mg/kg of the extract was administered to the animals for 15 days, after which seizures were induced by Maximum electro shock method and the duration of various phases of epileptic attacks were recorded and compared with the control animals. A significant ($P<0.01$ and $P<0.001$) reduction in the time taken for righting reflex (recovery) was noted in the experimental animals.

KEYWORDS:

Antiepileptic, Rosa damascena, Pandanus fascicularis and neuro protective.

INTRODUCTION

Epilepsy is a brain disorder characterized by convulsive seizures or loss of consciousness or both. Epilepsy is a major neurological disorder and up to 5% of the world population has epilepsy in their lifetime.

Drug therapy of epilepsy with currently available antiepileptic drugs (AED) is associated with side-effects, dose-related and chronic toxicity that involves virtually every organ system. Hazards of anticonvulsant therapy in pregnancy and teratogenic effects are well-known. Moreover, all the currently available AED have potential for adverse effects on cognition and behavior. This made man to search for alternative medicine from natural source.[1,2]

Medicinal plants used for the therapy of epilepsy in traditional medicine have been shown to possess promising anticonvulsant activities. The folk or traditional medicinal uses of plants represent "leads" that could short cut the discovery of modern medicines with novel structures, which can be much cheaper and less time-consuming. Several useful medicines derived from plants have been discovered from scientific investigation of traditional and folklore claims.[3,4,5] *Rosa damascena* mill L., known as Gole Mohammadi in is one of the most important species of Rosaceae family flowers. *R. damascena* is an ornamental plant and beside perfuming effect, several pharmacological properties including anti-HIV, antibacterial, antioxidant, Anticonvulsant, antitussive, hypnotic, antidiabetic, and relaxant effect on tracheal chains have been reported for this plant. This article is a comprehensive review on pharmacological effects of *R. damascena*.

Several components were isolated from flowers, petals and hips (seed-pot) of *R. damascena* including terpenes,

glycosides, flavonoids, and anthocyanins. This plant contains carboxylic acid, myrcene, vitamin C, kaempferol and quarcetin.

Pandanus fascicularis Linn. (family: Pandanaceae) is traditionally recommended by the Indian Ayurvedic medicines for treatment of headache, rheumatism, spasm, cold/flu, epilepsy, wounds, boils, scabies, leucoderma, ulcers, colic, hepatitis, smallpox, leprosy, syphilis, and cancer and as a cardiotonic, antioxidant, dysuric, and aphrodisiac. It contains phytochemicals namely, isoflavones, coumestrol, alkaloids, steroids, carbohydrates, phenolic compounds, glycosides, proteins, amino acids as well as vitamins and nutrients.

MATERIALS AND METHODS

Drugs and chemicals:

Diazepam (Calmpose inj., Ranbaxy), Phenobarbitone sodium (Luminal, Bayer AG), PTZ (Sigma Aldrich Chemical Co.) and INH (S.D. Fine-Chem. LTD) were used in this study.

Plant collection

The flowers petals of *Rosa damascene* and leaves of *Pandanus fascicularis* were collected from Warangal, Telangana, India. They were identified and authenticated by Prof. Ajmeera Ragan, Department of Botany, Kakatiya University, Warangal.

Preparation of poly-herbal formulation:

The flowers petals of *Rosa damascene* and leaves of *Pandanus fascicularis* were collected and they were shade dried at room temperature and 1 Kg of the dried flowers petals of *Rosa damascene* (500 gm) and leaves of *Pandanus fascicularis* (500 gm)were made in to coarse powder. The powder was passed through a No. 60 mesh sieve. Then methanol extract was prepared by following maceration method.[6]

Dosage:

The poly-herbal formulation was administered orally at doses of 250 mg/kg and 500 mg/kg in the form of suspension prepared in double distilled water containing carboxy methyl cellulose (1%, w/v, CMC).

Preliminary phytochemical investigations

To the 1gm of methanolic extract, 100ml of methanol was added and subjected to qualitative chemical tests for various phytoconstituents like terpenes, glycosides, flavonoids, and anthocyanins [7,8]. This plant contains carboxylic acid [9], myrcene [10], vitamin C, kaempferol and quarcetin [11], isoflavones, coumestrol, alkaloids, steroids, carbohydrates, phenolic compounds, glycosides, proteins, amino acids as well as vitamins and nutrients.

Pharmacological investigations

Animals

Adult Swiss albino rats (280-300 g) were used for this study. The animals were housed at 24°C ± 2°C and relative humidity 55 ± 5 with 12:12 h light and dark cycle. They were provided food and water *ad libitum*. The experimental protocol was approved by the Institutional Animals Ethics Committee of Aurobindo College of Pharmaceutical Sciences, Warangal, Telangana (CPCSEA no. **1761 / P0 / Ere / S / 14 / CPCSEA**).

Acute toxicity study:

Acute toxicity study was performed in accordance with OECD guidelines 423[7]. No adverse effect or mortality was detected in albino rats up to 3 gm/kg, p. o of poly-herbal formulation during the 24 to 72 hrs observation periods. For this period the rats were continuously observed for 5 hrs for any gross behavioral, neurological or autonomic toxic effect and lethally after 24 to 72 hrs.

Evaluation of antiepileptic activity

MES in rats

Four groups of six Swiss albino rats (280-300 g) of either sex were used. Group I received the vehicle, Group II and III received different doses (250 and 500 mg/kg, p.o.) of methanol extract of poly herbal formulation respectively. Group IV received the standard drug, diazepam at the dose of 3 mg/kg, p.o. The test was started 1 h after oral treatment with the extract or the vehicle or the standard. An apparatus with

corneal electrodes was used to deliver the stimuli. The intensity of the stimulus was dependent on the apparatus, e.g.: 45 mA, 50 Hz for 0.2 s has been used. Under these conditions all vehicle treated rats showed the characteristic extensor tonus. The animals were observed closely for 2 min. Disappearance of the tonic hind limb extensor was used as positive criterion. Percentage of inhibition of seizures relative to control was calculated.[12]

PTZ-induced convulsions in rats

Rats of either sex were randomly allotted to four different groups of six rats in each. Group I received the vehicle, Group II and III received poly herbal extract at the doses of 250 and 500 mg/kg, p.o. respectively. Group IV received the standard drug, phenobarbitone sodium at the dose of 40 mg/kg, i.p. Group I rats were administered with PTZ (75 mg/kg, i.p.) 1 h after vehicle. Group IV rats received PTZ 15 min after phenobarbitone sodium (40 mg/kg, i.p.). Group II and III received different doses of poly herbal plant extracts, p.o. 1 h before PTZ. Onset time as well as duration of convulsions were recorded.[13]

INH-induced convulsions in rats

Four groups of six Swiss albino rats (280-300 g) of either sex were used. Group I received the vehicle, group II and III received poly herbal plant extracts at the doses of 250 and 500 mg/kg, p.o. Group IV received the standard drug, diazepam at the dose of 4 mg/kg, i.p. 1 h after the administration of different extracts of poly herbal plant extracts, INH at a dose of 300 mg/kg, s.c. was administered. The rats were placed in isolated perplex chamber, during the next 120 min the latency of convulsions was recorded.[14]

Statistical analysis

The data were analyzed using one-way analysis of variance, followed by Dunnett's test. $P < 0.05$ was considered as statistically significant. The data are expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

Evaluation of antiepileptic activity

MES in rats

The average time of onset, duration of tonic hind limb extension (THLE) and percentages of inhibition of convulsions were presented in Table 1. Albino rats pretreated with poly herbal extract at the doses of 250 and 500 mg/kg, p.o. exhibited delay in the onset time of THLE when compared with the rats belonging to control group. Poly herbal extract treated rats also exhibited a decrease in duration of THLE when compared with the control group rats. Albino rats pretreated with poly herbal extract at doses 250 and 500 mg/kg were provided significant protection from convulsions induced by electroshock method in a dose-dependent manner ($P < 0.01$). Animals pretreated with poly herbal extract exhibited significant antiepileptic activity and more percentage inhibition of convulsions at the dose of 500 mg/kg when compared to diazepam treated animals.

Table 1: Effect of poly herbal formulation on Maximal electro shock -induced convulsion method in rats

Group	Treatment	Onset of THLE (Sec) Mean±SEM	Duration of THLE (Sec) Mean±SEM	% of inhibition of convulsions
Control	10 ml	1.13 ± 0.04	115.69 ± 0.22	-
PHE_1	250 mg/kg	1.97 ± 0.18	75.84 ± 0.37	34.78
PHE_2	500 mg/kg	2.31 ± 0.05	62.07 ± 0.13	46.08*
Diazepam	3 mg/kg	-	-	100**

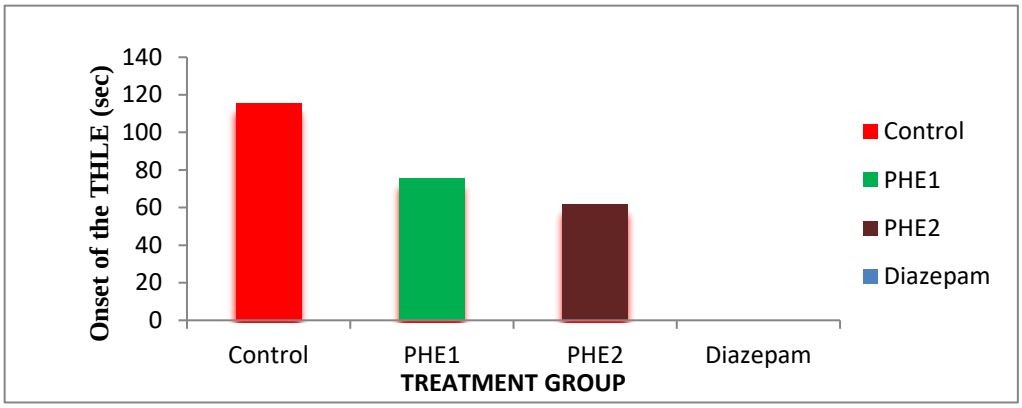

Fig 1(a): Onset of the THLE (sec) Mean ± SEM on different groups by maximal electroshock-induced convulsion method.

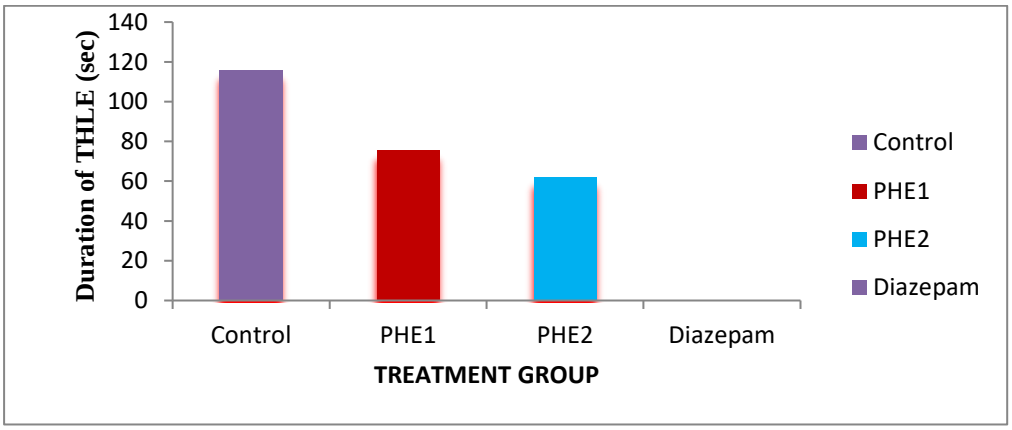

Fig 1(b): Duration of the THLE (sec) Mean ± SEM on different groups by maximal electroshock-induced convulsion method.

PTZ-induced convulsions in rats

The average time of onset, duration of convulsions and percentages of inhibition of convulsions were presented in Table 2. Poly herbal extract treated rats not only exhibited delay in the onset time of convulsions at the doses of 250 and 500 mg/kg, p.o. but also showed reduced duration of convulsions when compared with the control group rats. All the two doses of poly herbal extract afforded significant protection in a dose-dependent manner against convulsions induced by PTZ ($P < 0.01$). Animals pretreated with poly herbal extract at all the two doses exhibited significant antiepileptic activity and moderate percentage of inhibition of convulsions when compared with Phenobarbitone sodium treated animals.

Table 2: Effect of poly herbal formulation on PTZ-induced convulsion method in rats

Treatment Group	Dose	Onset of clonic convulsion (sec) Mean±SEM	Duration of Clonic convulsion (sec) Mean±SEM	% protection
Control	10 ml/kg	46.17 ± 2.84	187 ± 1.72	-
PHE_1	250 mg/kg	167.52 ± 0.96	79.01 ± 1.06	57.75
PHE_2	500 mg/kg	185.90 ± 0.31*	64.26 ± 1.35*	65*
Phenobarbitone sodium	40 mg/kg	0 ± 0**	0 ± 0**	100**

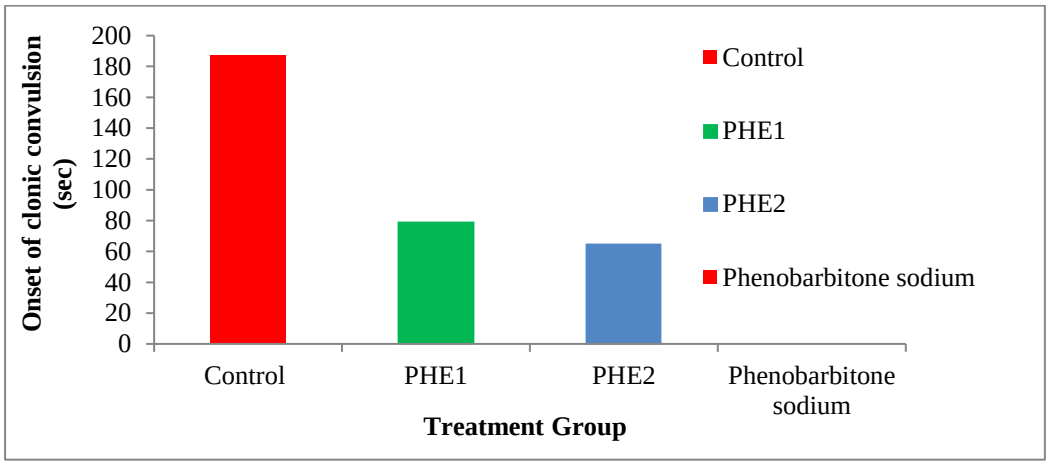

Fig 2(a): Onset of the clonic convulsion (sec) Mean ± SEM on different groups by PTZ -induced convulsion method.

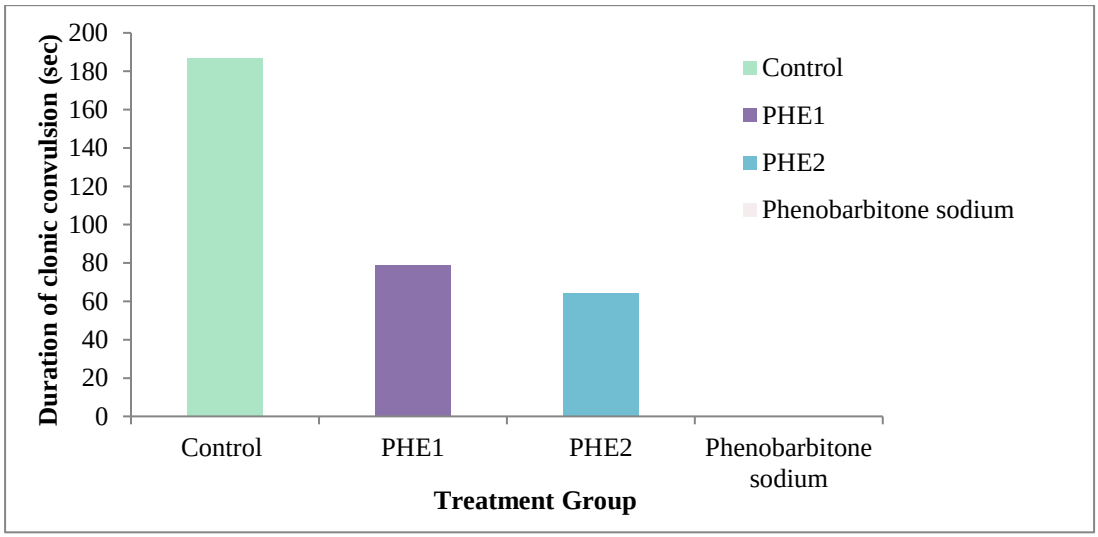

Fig 2(b): Duration of clonic convulsion (sec) Mean ± SEM on different groups by PTZ -induced convulsion method.

INH-induced convulsions in rats

The average latency of convulsions was presented in Table 3. All the three doses of poly herbal extract showed the latency of convulsions more than that of control and less than that of standard i.e. diazepam (4 mg/kg, i.p.) and although poly herbal extract delayed the latency of convulsions in a dose-dependent manner.

Table 3: Effect of poly herbal formulation on INH- induced convulsion method in rats

Treatment Group	Dose	Latency of convulsions (min) Mean±SEM
Control	10 ml	25.33 ± 0.16
PHE_1	250 mg/kg	38.63 ± 1.02
PHE_2	500 mg/kg	54.04 ± 1.37*
Diazepam	4 mg/kg	63.27 ± 0.97**

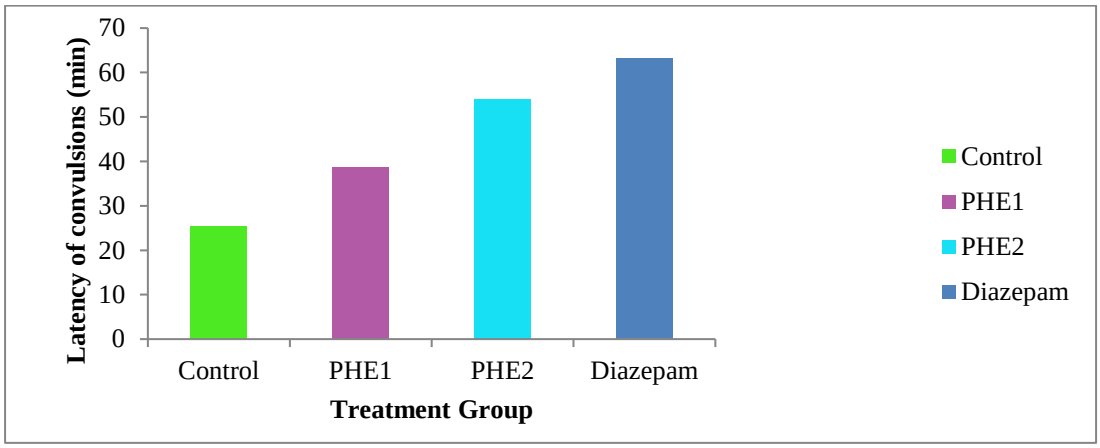

Fig 3: Latency of convulsions (min) Mean± SEM on different groups by INH -induced convulsion method.

Epilepsy is a group of chronic neurological disorders characterized by sporadic episodes of convulsive seizures, sensory disturbance, abnormal behavior and loss of consciousness or all of these symptoms resulting from a brain dysfunction or an abnormal discharge of cerebral neurons.[15]

Higher prevalence, lack of awareness, cultural and social stigma and non-availability of proper diagnostic and treatment facilities are among the major problems in the developing countries. Drug therapy of epilepsy with currently available AED is associated with side effects, dose-related and chronic toxicity that involves virtually every organ system. There is a pressing need for further research especially in the field of pharmacotherapy of epilepsy to find drugs. Search for anti-epileptic agents has made man turn to alternative sources, indigenous system of medicine.

Previous reports evidenced that many plants like *Hibiscus rosa*, *Cyperus articulatus*, *Delphinium denudatum*, *Carissa edulis* showed good protection against epilepsy.[16,17,18,19]

The essential oil of *R. damascena* in acute pentylenetetrazole (PTZ)-induced seizure in rats, delays the start of epileptic seizures and decrease the duration of tonic-clonic seizures (stage 4) [20,21]. In chronic model of PTZ-induced seizure, this plant also caused prolongation of latent periods before tonic-clonic generalized seizures [20].

Injection of essential oil 30 min before amygdale electrical kindling also reduced appearance of 1st, 2nd, 3rd, 4th, and 5th stages of seizure and could reduce the time after discharge duration. It is suggested that essential oil of *R. damascena* retarded the development of behavioral seizures in amygdale electrical kindling and possesses the ability to counteract kindling acquisition [21].

The mechanism(s) of these effects of *R. damascena* cannot be explained by the observed results. However, authors suggested that the flavonoids maybe involved in this effect. It is reported that flavonoids act on GABAergic system in the brain. Flavonoieds can also enhance the effect of benzodiazepines on GABA receptors. Other components of essential oil of *R. damascena* such as geraniol and eugenol have been shown to have antiepileptic effect [22]. However, the exact mechanistic effect of these compounds is unknown.

The effects of the essential oil of *R. damascena* as an adjunct in treatment of children with refractory seizures were also studied and showed a significant reduction in the mean frequency of seizures in patients using essential oil of the plant. Therefore, the essential oil of *R. damascena* has beneficial antiepileptic effect in children with refractory seizures.

Methanolic extract of *Pandanus fascicularis* is one such plant is used to treat epilepsy. The poly herbal

extract was evaluated for antiepileptic activity by three animal models involving gamma-amino butyric acid (GABA) ergic neurotransmission i.e. MES in mice, PTZ and INH-induced convulsions in rats.

The data obtained in the present study demonstrated for the first time that the poly herbal extract had significantly inhibited the convulsions induced by MES, PTZ and INH. GABA is known to be an important inhibitory neurotransmitter in the brain, whereas glutamate is the excitatory neurotransmitter. GABA acts on the GABA receptors and glutamate acts through the N-methyl-D-aspartate (NMDA) and non-NMDA receptors. Activation of these receptors modifies various voltage-gated Na^+, K^+, Ca^{++} and Cl^- ion channels and excites or inhibits the neuron. Abnormalities in the GABA system have been found in neurological and psychiatric diseases such as Huntingdon's chorea, anxiety, panic attacks, schizophrenia and epilepsy. One major factor in epileptogenesis seems to be a decreased function of $GABA_A$ synapses.[23]

The MES test in rats is a suitable model for grand mal epilepsy. MES test in rats is used primarily as an indication for compounds, which are effective in grand mal epilepsy. THLE are evoked by electric stimuli which are suppressed by antiepileptics. PTZ-induced convulsions in rats are a suitable model for petit mal epilepsy. PTZ is GABA antagonist. This assay has been used primarily to evaluate AED. Drugs which antagonize PTZ-induced seizures are generally useful in petit mal epilepsy. It has been indicated that PTZ-induced seizures can be prevented by drugs that reduce T-type Ca^{2+} currents, such as ethosuximide and also by drugs that enhance $GABA_A$ receptor-mediated inhibitory neurotransmission, such as benzodiazepines and Phenobarbital. INH can precipitate convulsions in patients with seizure disorders. INH is regarded as a GABA-synthesis inhibitor. Clonic tonic seizures are elicited in mice which are antagonized by AED.[24] The results of our study reveal that poly herbal extract significantly inhibited the convulsions induced by MES, PTZ and INH.

CONCLUSION

Based on the above investigations, it may be concluded that the methanolic extract of poly herbal extract exhibited significant antiepileptic activity. These findings justify the traditional use of this plant in the control and/or treatment of convulsions and epilepsy as the plant is unexplored yet. The presence of flavanoids may partially contribute the significant activity of methanolic extract of polyherbal formulation by enhanced GABAergic neurotransmission. Further detailed phytochemical investigations are required to identify the phytoconstituent/s responsible for the antiepileptic effect.

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