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EVALUATION OF ANTICONVULSANT ACTIVITY OF ETHANOLIC EXTRACT OF *DIMOCARPUS LONGAN LOUR* SEEDS IN MICE

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ABSTRACT

- **Background:** Epilepsy affects 1% of world population. Epilepsy has been well controlled with the currently available anti-epileptic drugs (AEDs). But most of the anti-epileptic agents have their own limitations like neurotoxicity and teratogenic effects. Hence there is continuous need for more effective newer AEDs.
- **Objective :-**

1. To evaluate the anticonvulsant activity of *Dimocarpus longan lour* seeds ethanolic extract in preventing maximal electroshock (MES) and pentylenetetrazole (PTZ) induced convulsions.
2. To compare its efficacy with standard drugs- phenytoin for MES and PTZ method.

- **Methods:**-48 male albino mice weighing 18-30g are selected and divided into 2 groups of 24 mice each – one group for MES and other group for PTZ method.

In MES method, seizures were induced via ear clip electrodes with a current of 50 mA for 0.2 seconds. Each mouse is pretreated with drugs (p.o.) one hour before MES test. The different groups include – Group C1 administered saline solution (10ml/kg), Group S1 administered phenytoin (25mg/kgI.P.), Group T1 administered ethanolic extract of *Dimocarpus longan lour* seeds (200 mg/kg), Group T2 administered ethanolic extract of *Dimocarpus longan lour* seeds (400mg/kg). **In PTZ method,** seizures were induced by giving PTZ 80 mg/kg SC. Each mouse is pretreated with drugs one hour before giving PTZ. The different groups include – Group C2 administered saline solution (10mg/ kg), Group S2 administered phenytoin (25mg/kg I.P.), Group T3 administered ethanolic extract of *Dimocarpus longan lour* seeds (200mg/kg), Group T4 administered ethanolic extract of *Dimocarpus longan lour* seeds (400mg/kg).

- **Statistical analysis**:-Statistical analysis was done by Student on ANOVA (Dunnett's‘t’ test).
- **Results:-** The ethanolic extract of *Dimocarpus longan lour* seeds at a dose of 400mg/kg has shown statistically significant anticonvulsant activity against both MES and PTZ convulsions and its anticonvulsant activity is similar to that of standard phenytoin (400mg/kg).
- **Conclusion**:- Our study demonstrates the anticonvulsant activity of ethanolic extract of *Dimocarpus longan lour* seed in mice. This plant can be a therapeutic potential to treat epilepsy in addition to conventional anticonvulsant drugs.

KEYWORDS: *Dimocarpus longan lour* seeds, anticonvulsant, Pentylenetetrazole and Maximal Electroshock model.

1. INTRODUCTION

Epilepsy is a chronic disorder of the brain that affects people worldwide. As per WHO, epilepsy is characterized by recurrent seizures, which are brief episodes of involuntary movement that may involve a part of the body (partial) or the entire body (generalized), and are sometimes accompanied by loss of consciousness and control of bowel or bladder function.

Epilepsy was one of the first brain disorders to be described. It was mentioned in ancient Babylon more than 3,000 years ago. The strange behavior caused by some seizures has contributed through the ages to many superstitions and prejudices. From Greek word attack, the word epilepsy is derived. In earlier times, People once thought that those with epilepsy were being visited by demons or gods. However, in 400 B.C., the early physician Hippocrates suggested that epilepsy was a disorder of the brain, and we now know that he was right.[2]

Epilepsy is a major neurological disorder and up to 5% of the world population develops epilepsy in their lifetime. The current therapy of epilepsy with modern antiepileptic drugs is associated with side effects, dose-related and chronic toxicity as well as teratogenic effects and approximately 30% of the patients continue to have seizures with current antiepileptic drug therapy.[4]

Traditional systems of medicines are popular in developing countries and up to 80% of the population relies on traditional medicines/folk remedies for their primary health care need. Hence, there is a need to discover an alternative agent from natural sources.

Longan (*Dimocarpus longan* Lour) is a subtropical evergreen tree belonging to the family Sapindaceae. It is found commonly in most of Asia, primarily in mainland China, Taiwan, Vietnam and Thailand. China, the main longan-producing country in the world, produced about 1,900 thousand tonnes of longan in 2015–2017.[7]

Longan seeds are used to prevent pain, hemorrhage, hernia, and skin diseases in Chinese folk medicine. The seeds act as antioxidant, prevent human colorectal carcinoma cells, and they contain Gallic acid, ellagic acid, and corilagin. The current study was undertaken to investigate the analgesic, CNS depressant and anti-diarrheal effects of some solvent extracts of the seeds of D. longan.[6]

2. MATERIAL AND METHOD

2.1 PLANT

- **Plant selection& collection:-** Selection of plant was based on traditional claim of medicines. Plant material was collection from local market in Indore.
- **Preparation of extraction:-** The plant material was identified and authenticated on the basis of macroscopic and microscopic characters as *Dimocarpuslongan*. The plant specimen voucher was deposited within the institute. The seeds which were collected from market were washed thoroughly with distilled water for extraneous matter. They were dried, ground and 500 grams of dried longan seed powder was extracted with 1 liter of absolute ethanol in soxhlet apparatus for 1 week. The pooled extracts were concentrated and then evaporated under vacuum and further evaporated by heating on 80°C water bath to dryness. This process yielded 80 grams of crude longan seed extract, which is stored in refrigerator in a closed container for further use.[11]
- **Phytochemical studies:-**Preliminary phytochemical tests were conducted on test extract to detect the presence of phytochemicals (Steroids, Alkaloids, Tannins, Saponins, Carbohydrates, Amino acid/ Protein, Gums and Mucilage by standard methods described in the Pharmacognosy text book of Trease and Evans.[12]

2.2 ANIMAL:- An adult albino mice (25-30gms) of either sex were used for the study. The animals were maintained in a well-ventilated room with 12:12 hour light/dark cycle in

polypropylene cages. Standard pellet feed and drinking water was provided ad libitum. Animals were acclimatized to laboratory conditions one week prior to initiation of experiments. The animals were divided into four groups, each consisting of six mice and were used in all sets of experiments.[5]

The experimental protocol was approved by the Institutional Animal Ethical Committee of our institute. **(Approval No: 1888/PO/Re/S/16/CPCSEA/2020/04)** and were strictly in accordance with the norms of Committee for the Purpose of Control & Supervision of Experiments on Animals (CPCSEA) New Delhi.

2.3 DRUGS AND CHEMICAL:-Drugs Diazepam, Phenytoin, Pentylenetetrazole (PTZ), and Picrotoxin (PCT) were purchased from Indore. All the drug solutions were freshly prepared by dissolving/suspending in normal saline for intraperitoneal (I.P.) administration. The solvents used were of analytical grade. Extract of dimocarpus seeds were administrated orally at dose of 200 & 400 mg/ kg.

2.4 ORAL TOXICITY STUDIES:-Acute toxicity study was performed according to OECD guideline No 423. The animals were fasted overnight and provided water only. The adverse effects taking place after oral or dermal administration of a single or multiple doses of a substance from 5, 50, 100, 200, 400, 1000, 2000mg/kg of body weight, given within 24 hours, or an inhalation exposure of 4 hours are called as acute toxicity. The reason behind this studies was to know the safe range of dose of drug, which helps to be used for further studies and also biologic activity of a test chemical, its mechanism of action and the acute oral toxicity of test substance must be carried out by single dose toxicity method on least number of animals. Observation parameters include were any change in skin and fur, eyes and mucous membranes, grooming, respiration, hyperactivity and behaviour pattern, tremors, convulsions, salivation, diarrhoea etc.

2.5 TREATMENT TESTING MODEL

I. **Maximal electroshock induced seizure:-** MES model was used for the evaluation of the anticonvulsant effect of *DLLSE*. Electro-Convulsiometer (Model No EC-02) was used for delivering an electric shock (150 mA for 0.2 seconds) with the help of corneal electrode to induce hind limb tonic extension (HLTE) in mice. DLLSE was administered at the dose of 200, and 400 mg/kg, orally while phenytoin (25 mg/kg, intraperitoneally) was

used as a standard drug. All the treatments were given 30 minutes before applying electric shock. Animals were divided into four groups, each group containing 6 mice.[3]

1. Group I received normal saline solution (10 ml/kg, orally).
2. Group II received the standard drug, Phenytoin (25 mg/kg, intraperitoneally).
3. Group III received *DLLSE* (200 mg/kg, orally).
4. Group III received DLLSE(400 mg/kg, orally).

The mice were then observed for 30 min for various phases of epilepsy like flexion, extensor, Clonus, stupor, along with the duration of each phase. Reduction or abolition of in the duration of hind limb tonic extensor (HLTE) phase was taken as a measure of protection against MES induced seizuresand was taken as a parameter for the evaluation of anti-epileptic activity.

II. Pentylenetetrazole (PTZ)-induced seizures:-PTZ-induced seizures model was used for the evaluation of anticonvulsant effects of *DLLSE*. PTZ (80 mg/kg) was injected intraperitoneally to induce convulsion in mice. *DLLSE* was administered at the dose of 200, and 400 mg/kg, orally while diazepam (5 mg/kg, intraperitoneally) was used as a standard drug. PTZ was administered 30 minutes after the drugs, and the animals were observed for 30 minutes after the administration of PTZ. The different parameters noted were the onset and duration of clonic convulsions, recovery/death i.e. mortality percentage. The anticonvulsant property was assessed by the ability to reduce the duration of clonic convulsions and increase the latency of seizures.[3]

1. Group I received normal saline solution (10 ml/kg, orally).
2. Group II received the standard drug, Phenytoin (25 mg/kg, intraperitoneally).
3. Group III received *DLLSE* (200 mg/kg, orally).
4. Group III received *DLLSE* (400 mg/kg, orally).

3. RESULT AND DISCUSSION

3.1 EXTRACTIVE YIELD OF EXTRACT

Percentage yield:- Dried powder of *Dimocarpus longan lour* seedswas extracted with ethanol using Soxhlet apparatus, percentage yield of extract was found to be 80% w/w.

3.2 PRELIMINARY PHYTOCHEMICAL SCREENING

Phytochemical screening of *Dimocarpus longan lour* seeds extract showed the presence of the various phytoconstituents like Flavonoids, saponins, tannins, alkaloids, carbohydrate,

steroids, Amino acid/ Protein, glycosides and Gums and Mucilage.

Table 1: Phytochemical screening of ethanolic extract of *D.longan* fruit seeds.

S.No	Phytochemicals	Result
1.	Flavonoid	+
2.	Steroid	+
3.	Alkaloids	+
4.	Tannins	+
5.	Carbohydrate	-
6.	Saponins	-
7.	Amino acids/ protein	+
8.	Glycosides	+
9.	Gum and Mucilage	-

3.3 ACUTE TOXICITY STUDY

The acute toxicity test was performed by using the ethanolic extract at dose levels 5mg/kg, 50mg/kg, 100 mg/kg, 200 mg/kg, 400mg/kg, 1000 mg/kg and 2000mg/kg. As it is a natural substance and is not expected to be particularly toxic. Hence when test animal were treated orally with a lower dose of 5mg/kg no sign of toxicity was observed and all animal were survived. Then 50mg/kg of test drug was administered orally no sign of toxicity was observed and all animal were survived. A lower dose of 100 mg/kg all animal were survived. A lower dose of 200 was given than a dose of 400 mg/kg was given all the animals were survived. A higher dose of 1,000 mg/kg was given, all animal were survived. 2000 mg/kg of test animal was administered orally and 3 animals were died.Hence *Dimocarpus longan lour seeds extract* was found to be safe at 1000 mg/kg.

Table 2: Acute toxicity study of *Dimocarpus longan lour* seeds.

No. of animal	Dose (mg/kg)	No. of animal survived
3	5	3
3	50	3
3	100	3
3	200	3
3	400	3
3	1000	3
3	2000	0

3.4 ANTICONVULSANT ACTIVITY

- **Maximal electroshock test:-** MES induced tonic seizures can be prevented either by drugs that inhibit voltage dependant Na+ channels such as Phenytoin, Valproate, Felbamate and Lamotrigine or by drugs that block glutaminergic excitation mediated

by then methyl-D-aspartate (NMDA) receptor, such as Felbmate. *Dimocarpus longan lour* seeds extract may follow any one of the above mechanism. The result of anticonvulsant effect of *Dimocarpus longan lour* seeds against MES and PTZ induced convulsions are shown in table 3 and table 4 respectively. The one way ANOVA analys is of the data observed indicated that ethanolic extracts of *Dimocarpus longan lour seeds* exhibited significant anti-seizure induced seizures. Control group animals exhibited hind limb tonic extension (HLTE) of 14.56 ± 0.67sec. after the delivery of an electroshock. Ethanolic extract at dose of 200 mg/kg body weight shown less effect on total duration of HLTE 8.25±0.47 while at the dose of 400, it significantly reduced the duration of HLTE to 6.83±0.27. The standard drug, Phenytoin at a dose of 25 mg/kg significantly reduced the duration of HLTE to 1.15±0.09. Statistically significant results P<0.01were observed with ethanolic extract at the dose of 200 and 400 mg/kg and standard drug, Phenytoin at a dose of 25 mg/kg.

Table 3: Effect of ethanolic extract of *Dimocarpus longan lour* seeds extracton MES induced seizures models.

S. No	Group	Flexion (Sec.)	Extensor (Sec.)	Clonus (Sec.)	Stupor (Sec.)	Recovery/ Death
1.	Control	9.6±0.37	14.56±0.67	17.76±0.61	52.3±1.75	Death
2.	Standard	6.08±0.32**	1.15±0.09**	8.6±0.46**	14.7±0.58**	Recovery
3.	Test Drug 200mg/Kg	8.06±0.30*	8.25±0.47**	15.03±0.73**	19.4±0.8**	Recovery
4.	Test Drug 400mg/Kg	7.38±0.55**	6.83±0.27**	11.88±0.44**	18.28±0.48**	Recovery

Values are expressed in Mean ± SEM (n = 6/group). One-way ANOVA, followed by Dunnett's multiple comparisons test; $*p < 0\ 05$, $**p < 0\ 01$, and $***p < 0\ 005$.

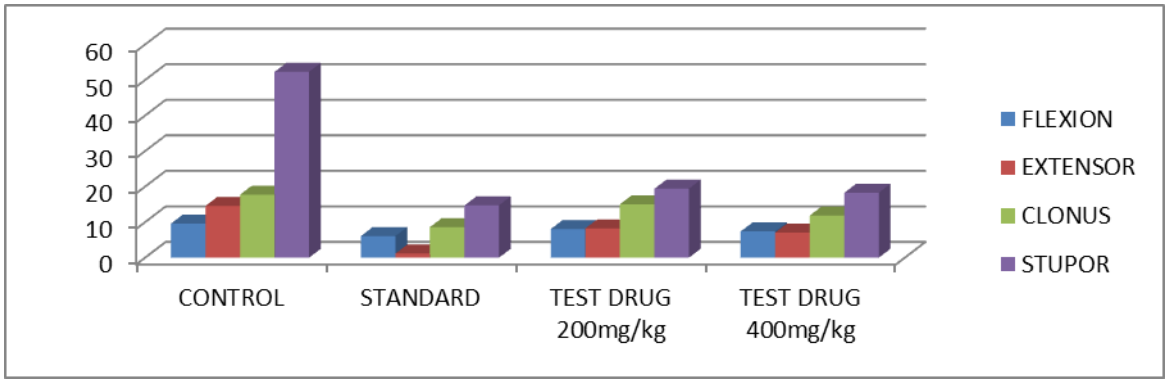

Figure 1:- Effect of Ethanolic extract of *Dimocarpus longan lour* seeds extract on MES induced seizures models.

Pentylenetetrazole Induced Seizures test:-In PTZ induced seizures, ethanolic extract dose 200 and 400 mg/Kg body weight exhibited delayed onset of clonus 105.4±1.77 and 155.9±1.75sec. respectively in comparison to control 52.06 ± 1.79 sec. As far as the duration of clonic convulsions is concern, ethanolic extract of *Dimocarpus longan lour seeds* extract at a dose 200mg/kg and 400mg/Kg exhibited significant (P<0.01) reduction in duration of clonic convulsions, 56.4±2.38 sec. and 51.3±2.18 sec. respectively in comparison tocontrol animals 89.3± 2.56 sec. The standard drug, Phenytoin at a dose of 25 mg/kg shows significant anticonvulsant activity. Further studies are necessary to elucidate the exact mechanism of action and the active principle responsible for above activity.

Table 4: Effect of Ethanolic extract of *Dimocarpus longan lour seeds* on PTZ induced seizures models.

S.No	Group	Onset of convulsion (Sec)	Duration of convulsion (Sec)	% Mortality	% Protection
1.	Control	59.61±1.52	89.31±2.56	50	0
2.	Standard	0±0**	0±0**	0	100
3.	Test Drug 200mg/kg	105.4±1.08**	56.4±0.97**	33	36.84
4.	Test Drug 400mg/kg	155.9±1.75**	31.33±0.89**	53.09	64.91

Values are expressed as the mean ± SEM (n = 6/group). One-way ANOVA, followed by Dunnett's multiple comparisons test; ∗p < 0 05, ∗∗p < 0 01, and ∗∗∗p < 0 005.

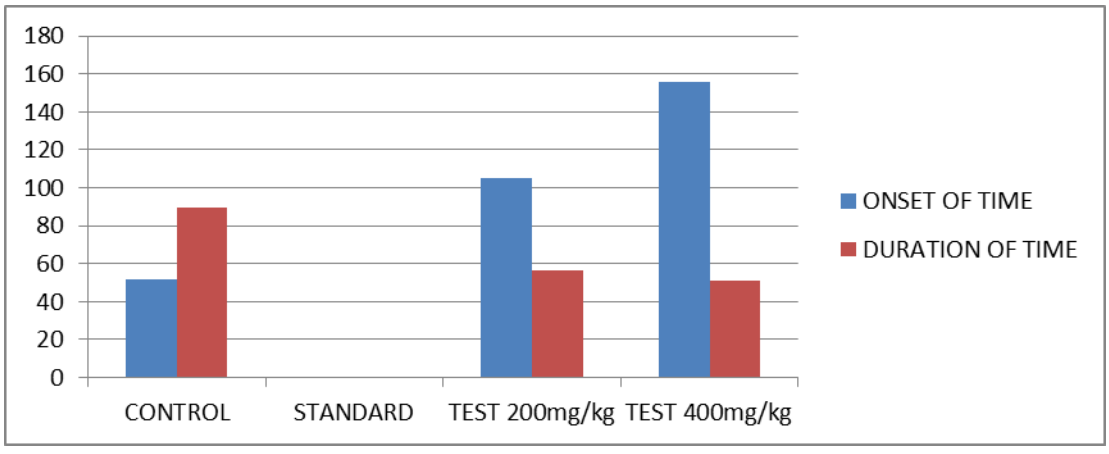

Figure 2: Effect of Ethanolic extract of *Dimocarpus longan lour seeds* on PTZ induced seizures models.

3.5 DISCUSSION

Epilepsy is a chronic disorder of the brain that affect peopleworldwide. Nearly about 50-80% of the patients with epilepsy are controlled with currently available antiepileptic drugs. But

these drugs cannot able to control seizures effectively in about10-20% of the patients. The treatment of epilepsy still remains inadequate even though new anticonvulsants are being developed. Furthermore the current therapy of epilepsy with modern antiepileptic drugs is associated with side effects, dose related and chronic toxicity as well as teratogenic effects. The anticonvulsants available are neither effective universally, nor safe. Due to long-term therapy with unwanted effects of many drugs the compliance with medication is very minimal. Traditional systems of medicines are popular in developing countries and upto80% of the population relies on traditional medicines/ folk remedies for their primary health care need. Hence, there is a need to discover an alternative agent from natural sources.

D. longan seeds uses as herbal medicine were used as traditional medicine for a long time; fruits of longan can relief of swelling and neural pain in traditional Chinese medicine. In the past reports found, each part of longan extracts including pulp, seed and peel were used to study bioactive compounds and their biological activities. Especially in longan seeds, the rich source of polyphenolic compounds such as gallic acid, corilagin, and ellagic acid that higher than pulp and peel. This study is to evaluate the anticonvulsant activity of ethanolic extract of seeds of DLLSE in MES and PTZ seizure-induced rats. MES and PTZ tests are the best-validated method for assessment of AED in human generalized tonic–clonic seizures and absence seizures, respectively, among the tests used for evaluation of anticonvulsant activity. Various studies shows thatthe active principle flavonoid having a crucial role in treatment of epilepsy. *D. longan* is rich in flavonoid. Since *D. Longan* has not been studied for its antiepileptic activity, the present study was aimed to evaluate the antiepileptic activity of ethanolic extract of *D. longan*.

The maximal electroshock induced convulsion in animals represents grandma type of epilepsy. The tonic extensor phase is selectively abolished by the drugs effective in generalized tonic clonic seizure. The result of the present study shows that theethanolic extract of *D. longan* at doses 200 and 400mg/kg significantly delayed the onset of HTLE and reduced the duration of HTLE. And also both doses completely abolished the phase of convulsion in MES induced convulsion models. In case of PTZ induced convulsion, the result of the present study shows that the ethanolic extract of *D. longan*, at doses 200 and 400 mg/kg significantly reduced the duration and also delayed the onset of convulsion when compared to controlgroup. PTZ may be exerting convulsant effect by inhibiting the activity of GABA at $GABA_A$ receptors. The results revealed that the *DLLSE* possess anticonvulsant

activity. The effect of *DLLSE* on oxidative stress in MES and PTZ induced convulsion was evaluated. *DLLSE* at doses 200 and400 mg/kg doses showed significant decrease in LPO level & GSH levels in brain tissue. *DLLSE* exhibit good antioxidant activity.

Epilepsy may develop because of an imbalance of neurotransmitters. In case of epilepsy, there may be abnormally high level of excitatory neurotransmitters (glutamate) that increase neuronal activity, while abnormally low level of inhibitory neurotransmitters (GABA) that increase neuronal activity in the brain. Hence, GABA hypo-activity and glutamate hyperactivity can enhance anepileptic seizure. In epileptic foci, GABA hypo-activity, which reduces the activity of dopaminergic neurons through a presynaptic effect through $GABA_A$ receptors. At low doses, NA can enhance epileptic seizures, where as at high doses, as a protective effect on seizures. The result of the present study shows that *DLLSE* significantly increase the level of inhibitory neurotransmitter GABA and also showed significant increase in the levels of DA, NA and 5-HT when compared to control group.

In our study, it was found that treatment with *DLLSE* extracts (200 and 400mg/kg) in mice significantly reduced THLE in MES-induced seizure model. MES-induced seizures are abolished by the drugs that block voltage-gated Na^+ channels such as phenytoin. Protection of *DLLSE* extract against THLE indicates that the drug possesses the ability to inhibit or abolish the spread of seizures within the brain suggesting the presence of an anticonvulsant compound in the extract. Similarly, it was found that treatment with *DLLSE* extracts (200 and 400mg/kg) significantly prolong the mean duration of seizure latency in PTZ seizure model. PTZ induced convulsions are prevented by the drugs that block T-type Ca^{2+} current in thalamus like sodium valproate or thedrugs which possess gamma-aminobutyric acid ($GABA_A$)agonistic like diazepam. Protection of DLLSE against PTZ induced seizure suggests a possible interaction with GABA-ergic neurotransmission indicating the presence of an anticonvulsant compound in the extract. Hence, the result indicates that *DLLSE* have good anticonvulsant activity.

4. CONCLUSION

Epilepsy is a neurological disorder that affects a wide range of people through out the world. Approximately 30% of the patients continue to have seizures with current antiepileptic drug therapy. Natural products from folk remedies have contributed significantly in the discovery of modern drugs and can be an alternative source for the discovery of antiepileptic drugs with

novel structures and better safety and efficacy profiles. The ethanolic extract of *Dimocarpus longan lour* seeds delay the onset and reduced the duration of convulsion in MES and PTZ induced convulsion models and can be used as an adjuvant therapy against cognitive deficit in convulsions. Also *DLLSE* significantly increased the level of inhibitory neurotransmitter GABA and also showed increase in DA, NA and 5-HT levels. Hence it can be concluded that the *DLLSE* possesses good anticonvulsant activity. Further studies are needed to explore the mechanism as well as the active principle responsible for the anticonvulsant activity of *Dimocarpus longan lour* seeds.

In conclusion, the results suggest that the ethanolic extract of *Dimocarpus longan lour* seeds possesses anticonvulsant activity which can be compared with the standard phenytoin in electrically and chemically induced epileptic animal models. Further studied are required to elucidate the exact mechanism by which this plant acts as an anticonvulsant agent.

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