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CNS Activities of Hydroalcoholic Extract of *Lawsonia inermis* Linn. Root

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ABSTRACT
Lawsonia inermis L. (Lythraceae), popularly known as henna, is a small tree or shrub. Its root has been used in Philippian alternative medicine for hysteria and nervous disorders, but to our best knowledge no scientific study has been conducted on these activities. Therefore here an attempt is done to screen anxiolytic and antidepressant activity of hydroalcoholic extract of *Lawsonia inermis* L. root in albino mice. The extract, administered orally, was able to increase open arm exploration in elevated plus maze and increase nose poking rate in hole board test for anxiolytic activity.The same extract increase immobility time of mice subjected to forced swim test and tail suspension test for antidepressant activity. The screening test for antidepressant effect of the drug is not encouraging or negative. Therefore the data obtained from the study allows us to suggest *Lawsonia inermis* root as an anxiolytic agent but not as an antidepressant. Preliminary phytochemical screening revealed the presence of carbohydrates, sterols, terpenoids, flavanoids, saponins and coumarins which are responsible for these pharmacological effects.
Keywords: *Lawsonia inermis* L., anxiety and depression

INTRODUCTION

The anxiolytics, antipsychotic, mood-stabilizing, and antidepressant agents are medications, intended to sedate, stimulate or modifying mood, thinking or behaviour, have a remarkable impact on psychiatric practice. The availability and use of these medications has grown since the late 1950s.Over 50% of adult population will experience some type of psychological disorder at some point in their life. Some degree of these disorders are part of normal life. Treatment is needed when it disproportionate to the situation and become excessive.The proposed study was aimed on anxiety and mood disorders.One of the main reasons behind these disorders is fluctuation of central neurotransmitters like GABA, glycine, norepinephrine, dopamine and serotonin. Drugs used for the treatment of psychiatric disorders act on these neurotransmitter receptors and produce desirable effect. Currently, most widely prescribed anxiolytics are benzodiazepines and antidepressants are MAO & serotonin inhibitors. However these modern medicine have some side effects. Ataxia, confusion, motor and cognitive impairment are the main side effect associated with benzodiazepines. Antidepressant produce side effects like gastrointestinal distress, insomnia, headache etc. Plant medicines are advantageous as being safer and less damaging to the human body than the synthetic drugs. Therefore the development of natural remedies with less side effects become acceptable.

In Philippian traditional medicine root of *Lawsonia inermis* L. (Lythraceae), popularly known as henna, administered orally for the treatment of hysteria and other CNS disorders.Some activities like abortifacient[1] and anticancer [2] of root of *L. inermis* were already reported. But there is no scientific report for its CNS effects. So the present study was undertaken to investigate whether the administration of hydroalcoholic extract from root of *Lawsonia inermis* L. produce behavioural modification in mice. Anxiolytic were assessed in the elevated plus maze & hole board test. Antidepressant effects were assessed by forced swim test and tail suspension test.

MATERIALS AND METHODS

Plant materials

Preparation of 80% ethanolic extract of *Lawsonia inermis* root.

The collected roots were washed with water, cut into small pieces and dried under the shade. The dried plant material was milled to a fine powder using the commercial laboratory blender. The dried powder was extracted in a Soxhlet extractor with hydroethanol (80:20%).The extraction was continued for 72 hour or until the solvent in the thimble was cleared. Air dried the extract and was then stored in a desiccator & used for further investigations. The percentage yield was 4.7%

Preliminary phytochemical screening

Phytochemicals are chemical compounds that occur naturally in plants. The presence of various phytoconstituents like alkaloids, carbohydrates, sterols, triterpenoids, cardiac glycosides, flavanoids, saponins and tannins were determined by the standard qualitative methods.[3, 4]

Drugs and chemicals

Diazepam (Roche laboratories), Imipramine (Novartis Ltd.) were used as standard drugs for anxiolytic and antidepressant activity respectively. Hydroalcoholic extract of *L. inermis* 200 & 400 mg/kg was suspended in 2% tween 80 .The standard drugs were dispersed in distilled water containing vehicle(2% tween 80). All drugs were given orally in a volume of l ml /100g body weight of mice using an oral feeding tube. Fresh drug solutions were prepared on each day of the experiment.

Experimental animals & exposure conditions

The animal albino Wistar mice were purchased from Animal house, Gov. Veterinary College Mannuthy. The animals were fed with rat feed and water *ad libitum.* They were housed in clean poly propylene cages, under identical conditions of food, water and temperature. Male and female animals were kept in separate cages. They were exposed to 12 hours, light-dark cycle and the relative humidity was in the range of 61-76% and temperature range was 15-25^{0}C. All procedures were performed according to CPCSEA guidelines after proper approval from the Institutional Animal Ethics Committee (IAEC, proposal no. SJCP/IAEC/04/2014), St. Joseph's College of Pharmacy, Cherthala.

Selection of dose of the extract

Dose of the extract was selected byacute oral toxicity studies. Manjula et al; (2012) and other researchers/literatures already reported that alcoholic extract of root was nontoxic up to dose 2000 mg/kg body weight and extract did not produce any mortality.[5] Hence, 1/10th (200mg/kg) and 1/5th (400mg/kg) of this dose were selected for further studies.

Behavioural paradigms

Anxiolytic activity

Elevated plus maze test

The test has been proposed for selective identification of anxiolytic and anxiogenic drugs. Anxiolytic compounds, by decreasing anxiety, increase the open arm exploration time; anxiogenic compounds have the opposite effect. The apparatus consist of two open arms (16 x 5 cm for mice and 50 x 10 cm for rats), two closed arms (16 x 5 x 12 cm for mice and 50 x 10 x 40 cm for rats), and an open roof with the entire maze elevated (25 cm for mice and 50 cm for rats) from the floor.

Procedure:

Albino mice (20-25 gm) were taken and divided into four groups, each group comprised of 6 animals. Animals were fasted 18 h prior to the experiment. **Group I** - control and was given vehicle (2% tween 80), p.o, **Group II**- standard and was given standard drug diazepam (2mg/kg, orally), dispersed in distilled water containing vehicle. **Group III & IV** - test groups and were given *L. inermis* extract 200 and 400mg/kg, p. o respectively. Prior and after 60 minutes of drug administration the animals were placed individually in the centre of the maze, head facing towards open arms and the stop watch was started and following parameters were notedfor 5 min. a) First preference of mice to open and closed arm, b) Number of entries in open and closed arms (an arm entry defined as the entry of fourpaws into the arm) c) Average time each animal spends in each arm. During the entire experiment, the animals were allowed to socialize. Every precaution was taken to ensure that no external stimuli could invoke anxiety in the animals.

Hole board test

It was proposed for the evaluation of curiosity. Number of nose poking into holes is considered as evaluating parameter.The hole-board has a sizeof 40 × 40 cm. Sixteen holes with a diameter of 3 cmeach are distributed evenly on the floor. The board iselevated to the height 25 cm, so that the mouse poking its nose into the hole does not see the bottom.

Procedure

Albino mice of weight between18 and 22 g are used.Animals were divided into four (I-IV) groups, each group comprised of 6 animals. **Group I** - a negative control and was given vehicle (2% tween 80), p.o. **Group II**- positive control and was treated with standard drug, diazepam (2mg/kg, orally), dispersed in distilled water containing vehicle. **Group III & IV** - test groups and were given *L. inermis* 200 and 400 mg/kg respectively. At the start of the test, mouse was placed in the edge of the board. The number of nose poking into the holes during a 5 minutes period is taken as the measurement. It was measured by visual observation prior and 60, 120 and 180 minutes after drug administration. [6-11]

Antidepressant activity

Forced swim test

Behavioural despair was proposed as a model to test for antidepressant activity. It was suggested that mice or rats forced to swim in a restricted space from which they cannot escape. Then they produced to a characteristic behaviour of immobility. This behaviour reflects a state of despair which can be reduced by several agents which are therapeutically effective in human depression. A vertical Plexiglas cylinder (height: 40 cm; diameter: 18 cm, containing 15 cm of water maintained at 25 °C) was used as apparatus.

Procedure

Albino mice 20-30g were used. Animals were divided into four groups, each group comprised of six animals.**Group I** – Control, received 2% tween 80, p. o, **Group II** – Standard- was treated with Imipramine 15 mg/kg, p. o ,**Group III** & **IV**– test groups and were treated with *L. inermis* 200 & 400 mg/kg, p. o, respectively. Prior and after 60, 120 & 180 minutes of drug administration mice were individually forced to swim inside a vertical plexiglas cylinder. Mice placed in the cylinders for the first time are initially highly active, vigorously swimming in circles, trying to climb the wall or diving to the bottom. After 2–3 minutes activity begins to subside and to be interspersed with phases of immobility or floating of increasing length. After 5–6 minutes immobility reaches a plateau

where the mice remain immobile for approximately 80% of the time. After swimming session the mice were removed and allowed to dry in a heated enclosure (32 °C) before being returned to their home cages. They are again placed in the cylinder 24 h later and the total duration of immobility is measured during a 5 minutes test. An animal is judged to be immobile whenever it remains floating passively in the water in a slightly hunched but upright position, its nose just above the surface.

Tail suspension test

The "tail suspension test" has been described as a facile means of evaluating potential antidepressants.

Procedure

Male albino mice weighing 20–25 g are used preferentially. Animals were divided into four groups, each group comprised of six animals. **Group I** – Control, received 2% tween 80, p. o **Group II** – Standard group, was treated with Imipramine 15mg/kg, p. o ,**Group III & IV** – received *L. inermis* 200 & 400 mg/ kg, p. o, respectively. Animals were transported from the housing room to the testing area in their own cages and allowed to adapt to the new environment for 1hourbefore testing. For this test the mice were suspended on the edge of a shelf 58 cm above a table top by adhesive tape placed approximately 1 cm from the tip of the tail. The duration of immobility is recorded prior and after 60,120 & 180 minutes for a period of 5 minutes. Mice are considered immobile when they hang passively and completely motionless for at least 1 minutes.[12-19]

Statistical evaluation

The results were expressed as mean ± SEM. Statistical analyses of all the data obtained were evaluated using one-way ANOVA followed by Dunnett's post –hoc multiple comparison test with SPSS Program ;Version 20. All the results were also expressed as graph by Graph Pad Prism software (V.5). P values < 0.05 were considered as statistically significant.

RESULTS

Phytochemical screening of plant extract

The preliminary phytochemical studies of extract revealed the presence of carbohydrate, steroids, terpenoids, flavanoids, saponins, tannins & coumarins and absence of alkaloids. The obtained results were shown in table 1.

Table1: Preliminary phytochemical screening of *L. inermis*

CONSTITUENTS	RESULT
Alkaloid	-
Carbohydrate	+
Sterols & terpenoids	+
Cardiac Glycosides	-
Flavanoids	+
Saponins	+
tannins	+

Key: + (positive) means Present, - (negative) means absent

Elevated plus maze test

The open arm exploration effect of extract and diazepam were analysed by Dunnett't' test. The results showed maximum effect at 120 minutes. So the statistical evaluation were done by using this data. The standard drug diazepam and *L. inermis* root extract 400 mg/kg significantly increased (P<0.001) percentage of time spent in open arm and these drugs significantly decreased (P<0.001) percentage of time spent in closed arm when compared to control. *L. inermis* extract 200mg/kg was also significantly increased (P<0.01) percentage time spent in open arm and significantly decreased (P<0.01) these parameter in closed arm when compared to control. These results were shown in table 2 & 3.

The effect of extract and diazepam on percentage of entries in arms were shown in table 4 & 5.The results showed that diazepam and 400 mg/kg extract increased the percentage of open arm entries significantly at P<0.001 and P<0.01 respectively and these significantly decreased the percentage of closed arm entries significantly at P< 0.001 and P<0.05 respectively. *L. inermis* 200 mg/kg significantly decreased (P<0.5) percentage of open arm entries but it didn't show any significant change in percentage of closed arm entries.From these two results we can say that *L.inermis* (200 & 400 mg/kg) have open arm exploratory activity.

Treatment groups	Time spent in open arm (seconds)				Percentage increase of time spent in open arm at 120 minutes
	Pre treatment	Post treatment			
		60 minutes	120 minutes	180 minutes	
Control	30.33 ±0.49	29.50 ±0.34	30.33 ±0.33	29.83±0.40	0.6 ±1.83
Standard (Diazepam 2mg/kg)	55 ± 4.02	69.50 ±6.35	80.50 ±6.58	74.50 ±5.68	29.07 ±3.53***
L. inermis extract 200mg/kg	40.17 ±5.19	44 ± 5.13	48.50 ±3.37	47.83 ±4.38	17.05 ±3.40 **
L. inermis extract 400mg/kg	49.33 ±3.46	60.67 ±2.92	68.33 ±4.42	64 ± 3.37	26.27 ±1.72***

Table 2: Effect of hydro alcoholic extract of *Lawsonia inermis* root (200 & 400 mg/kg, p. o) and diazepam (2mg/kg) on time spent in open arm in elevated plus maze

All values are expressed as mean ± SEM, n=6, using one- way ANOVA followed by Dunnett 't' test.*P< 0.05, ** P< 0.01, *** P< 0.001 – compared to normal control group

Treatment groups	Time spent in closed arm (seconds)				Percentage decrease of time spent in closed arm at 120 minutes
	Pre treatment	Post treatment			
		60 minutes	120 minutes	180 minutes	
Control	95.17 ±4.01	94.33 ±3.58	94.50 ±3.25	95.50 ±3.35	0.53 ± 0.86
Standard (Diazepam 2mg/kg)	95.67 ±1.91	83.83 ±1.58	76.17 ±2.0	81.83 ±5.33	20.20 ±2.06***
L. inermis extract 200mg/kg	79.17 ±3.08	75.17 ±1.64	71.17 ±2.06	74.67 ±2.53	7.80 ±0.87**
L. inermis extract 400mg/kg	100.67 ±1.15	94.17 ±2.04	88 ±1.57	87.17±3.29	12.59 ±0.96***

Table 3: Effect of hydro alcoholic extract of *Lawsonia inermis* root (200 & 400 mg/kg, p. o) and diazepam (2mg/kg) on time spent in closed arm in elevated plus maze.

All values are expressed as mean ± SEM, n=6, using one- way ANOVA followed by Dunnett 't' test.*P< 0.05, ** P< 0.01, *** P< 0.001 – compared to normal control group

Treatment groups	Number of entries in open arm				Percentage increase in open arm entries at 120 minutes
	Pre treatment	Post treatment			
		60 minutes	120 minutes	180 minutes	
Control	3.67 ±0.33	4.17 ±0.31	3.83 ±0.31	3.5 ±0.22	3.33 ± 4.26
Standard (Diazepam 2mg/kg)	2.83 ± 0.40	4 ± 0.52	8.67 ± 0.33	4.67 ±0.33	67.87 ± 3.50 ***
L. inermis extract 200mg/kg	2.50 ± 0.43	3.33 ± 0.42	4.83 ± 0.48	3.33 ± 0.56	38.36 ± 4.17 *
L. inermis extract 400mg/kg	2.17 ± 0.48	2.50 ± 0.56	4.17 ± 0.54	2.83 ± 0.31	49.45 ± 3.81**

Table 4: Effect of hydro alcoholic extract of *Lawsonia inermis* root (200 & 400 mg/kg, p. o and diazepam (2mg/kg, p. o) on open arm entries in elevated plus maze

All values are expressed as mean ± SEM, n=6, using one- way ANOVA followed by Dunnett 't' test.*P< 0.05, ** P< 0.01, *** P< 0.001 – compared to normal control group

Treatment groups	Number of entries in closed arm				Percentage decrease in closed arm entries at 120 minutes
	Pre treatment	Post treatment			
		60 minutes	120 minutes	180 minutes	
Control	5.17± 0.48	5 ± 0.36	4.83±0.48	5.17 ±0.31	5.28±1.15
Standard (Diazepam 2mg/kg)	8.17±0.48	6.83 ± 0.48	4.83±0.54	4.83 ±0.31	40.74±3.27 ***
L. inermis extract 200mg/kg	9.50±0.76	6.83±0.31	5.83 ±0.31	8 ± 0.73	15.80±3.58
L. inermis extract 400mg/kg	9 ± 0.52	7 ± 0.58	6.17±0.48	5.66±0.42	31.77±4.14 **

Table 5: Effect of hydro alcoholic extract of *Lawsonia inermis* root (200 & 400 mg/kg, p. o) and diazepam (2mg/kg, p. o) on closed arm entries in elevated plus maze

All values are expressed as mean ± SEM, n=6, using one- way ANOVA followed by Dunnett 't' test.*P< 0.05, ** P< 0.01, *** P< 0.001 – compared to normal control group.

Treatment groups	Number of nose poking			
	Pre treatment	Post treatment		
		60 minutes	120 minutes	180 minutes
Control	10.50 ± 1	11.50 ± 0.67	11.83 ± 0.79	10.83 ± 1
Standard (Diazepam 2mg/kg)	12 ± 1.06	23.67± 1.84	32.83 ± 1.67 ***	1. 2.22
L. inermis extract 200mg/kg	9.83 ± 0.79	18.17 ± 1.14	18.17 ±4.19 *	17.67 ± 4.17
L. inermis extract 400mg/kg	11.83 ± 1.0	20.83 ± 1.78	27.83 ± 2.06 ***	25.33 1.43

Table 6: Effect of hydro alcoholic extract of *Lawsonia inermis* L. root (200 & 400 mg/kg, p. o) and diazepam (1mg/kg, p. o) on nose poking behaviour

All values are expressed as mean ± SEM,n=6, using one- way ANOVA followed by Dunnett 't' test.*P< 0.05, ** P< 0.01, *** P< 0.001 – compared to normal control.

Treatment groups	Duration of immobility (seconds)			
	Pre treatment	**Post treatment**		
		60 minutes	**120 minutes**	**180 minutes**
Control	85 ± 1.73	84.50 ± 1.92	83.67 ± 1.52	85.50 2.05
Standard (Imipramine 15mg/kg)	89.50 ± 1.23	65.17±1.97	49.33±0.67	48.83 1.0 ***
L. inermis extract 200mg/kg	83.50 ± 2.14	93.17 ± 2.07	98.17 ± 2.18	2.52
L. inermis extract 400mg/kg	85.50 ± 5.69	102.33 ± 7.78	107.83 ± 7.91	113.00 ± 4.10 *

Table 7: Effect of hydro alcoholic extract of *Lawsonia inermis* root (200 & 400 mg/kg, p. o) and Imipramine (15mg/kg, p. o) on immobility time in forced swim test.

All values are expressed as mean ± SEM,n=6, using one- way ANOVA followed by Dunnett 't' test.*P< 0.05, ** P< 0.01, *** P< 0.001 – compared to normal control

Treatment groups	Duration of immobility (seconds)			
	Pre treatment	**Post treatment**		
		60 minutes	**120 minutes**	**180 minutes**
Control	67.50 ± 3.04	67.50 ± 2.75	68.16 ± 2.81	66.83 ± 2.79
Standard (Imipramine 15mg/kg)	85.83 ± 3.50	52.83 ± 2.98	45.50 ± 2.84	42.17 ± 3.28***
L. inermis extract 200mg/kg	50.83 ± 1.17	65.67 ± 2.18	69.33 ± 3.95	69.00 ± 4.31
L. inermis extract 400mg/kg	67.33 ± 1.96	80.83 ± 4.96	85.33 ± 4.26	93.33 ± 4.67 **

Table 8: Effect of hydro alcoholic extract of *Lawsonia inermis* L. root (200 & 400 mg/kg, p. o) and Imipramine (15mg/kg) on immobility time in tail suspension test

All values are expressed as mean ± SEM for six animals in each group using one- way ANOVA followed by Dunnett 't' test.*P< 0.05, ** P< 0.01, *** P< 0.001 – compared to normal control93.33 ± 4.67

Hole board test

The effect of L. inermis (200 & 400mg/kg) and diazepam (2mg/kg) on nose poking behaviour in hole board test with mice is shown in table 6. This data shows a maximum effect at 120 minutes. So the statistics were done using data at 120 minutes. Diazepam (2mg/kg) and L. inermis 400 mg/kg treated groups showed increase in nose poking rate significantly at P<0.001, but L. inermis 200 mg/kg significantly increase this behaviour at P< 0.05 when compared to nose poking rate of normal control group.

Despair swim test

The results showed a maximum effect at 180 minutes. So the statistical evaluation was done by using this data. The animals treated with standard drug (Imipramine 15 mg/kg) showed significant (P<0.001) decrease in immobility time while the animals treated with L .inermis 400 mg/kg significantly increased (P<0.05) the immobility time when compared to control group. There were no significant difference between L. inermis 200mg/kg treated group's and control group's immobility time. The results are shown in table 7.

Tail suspension test

The effect of extract on immobility time in tail suspension test was shown in table 8.This showed a maximum effect at 180 minutes. So the further evaluation was done using the data at 180 minutes. The animals treated with standard drug (Imipramine 15 mg/kg) decreased immobility time significantly at P< 0.001.While the animals treated with L. inermis 400mg/kg significantly increased (P<0.01) immobility time. There were no significant difference between L. inermis 200mg/kg treated group's and control group's immobility time.

DISCUSSION AND CONCLUSION

Here two main CNS activities were evaluated, anxiolytic and antidepressant activities of hydroalcoholic extract of *Lawsonia inermis* root. In this work anxiolytic effects were screened by elevated plus maze test and hole board test.

Elevated plus maze test is a widely used animal model for testing anxiolytic activity. The notable parameters in this test are percentage of time spent in open & closed arm and percentage number of entries in these two arms. Anxiolytics increase percentage time spent and percentage number of entries in open arm while the anxiogenic decrease these parameters. The results of the present work demonstrate that L. inermis root extract (200 & 400 mg /kg, p.o) exhibited anxiolytic effect similar to that of reference drug diazepam 2mg/kg, p.o.L.inermis extract 400 mg /kg significantly increased percentage time spent & entries into open arm and it significantly decreased these parameters in closed arm while L. inermis extract 200 mg/kg doesn't significantly decreased the percentage entries in closed arm. The results showed that more anxiolytic activity is produced by L. inermis extract 400 mg/kg.

The anxiolytic activity of extract can be confirmed by hole board test. In this test the expression of anxiolytic state may be reflected by an increase in nose poking behaviour. The results showed that L.

inermis extract 200 & 400mg/kg significantly increase nose poking behaviour and these effects were similar to mice treated with reference drug diazepam1mg/kg. L. inermis extract 400mg/kg showed higher significant effect than L. inermis extract 200 mg/kg. This may be indicated that higher dose of L. inermis extract produce more anxiolytic effect.

In this study the antidepressant activity of L. inermis was evaluated by forced swim test and tail suspension test where duration of immobility is considered as screening parameter. Antidepressants decreased this parameter. Here immobility of extract and reference drug compared with control group's effect. Results indicated that the immobility time of extract treated rats is increased significantly with increase in dose (400 mg/kg). There were no significant difference between L. inermis extract 200mg/kg treated group's and control group's immobility time. Based on these result we can say that L. inermis extract has no antidepressant activity at 200 & 400 mg/kg and it produce mild CNS depression at 400mg/kg.

Many researchers suggest that the plants containing flavanoids, saponins, tannins, steroids, triterpenes and monoterpenes have pharmacological activities in CNS. They reported that saponins and flavanoids possess anxiolytic activity. Triterpenoids and monoterpenoids produce sedative properties.20-23The phytochemical screening of present study reports the presence of these constituents. These findings raised the possibility that the CNS effect of the extract may be exerted by the phytoconstituents present in the tested plant extract.

The result of the study indicated that L. inermis extract has anxiolytic activity but no antidepressant activity. These activities are in a dose dependent manner. It is well known that most of the antianxiety drugs like benzodiazepines are mild CNS depressants or it produce sedation as side effect. So it is suggested that CNS activity of L. inermis may be similar to that of benzodiazepines which targets GABAA receptors. The CNS depressant effect of extract is not confirmed because in hole board test and elevated plus maze test the extract didn't show any change in locomotion. Therefore further studies using other behavioural measures such as pentobarbital induced sleeping test and rota rod test are needed to confirm its depressant effect.

Data obtained from various studies allows us to propose *Lawsonia inermis* L. root as anxiolytic agent and this validates its use in Ayurvedic & Philippians traditional medicine. The screening test for antidepressant effect of the drug is not encouraging or negative. The results are similar to anxiolytic activity with mild CNS depressant properties of various antianxiety agents like benzodiazepines. Further studies on Lawsonia inermis's other extracts and its isolation will help us to identify the possible phytochemicals involved, and to establish a clear mode of action for the effects produced. The present study revealed the basis of some traditional uses of the drug and ithelpsthe scientific people who search for nature remedies.

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