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Research Article

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Evaluation of Sedative Activity of Methanol Leaf Extract of Ceiba Pentandra Linn (Malvaceae) Using Mice

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Abstract

Background: *Insomnia and other associated disorders have been traditionally managed using leaves of Ceiba pentandra (Malvaceae).*

Methods: *In this study, sedative and anxiolytic properties of methanol leaf extract of Ceiba pentandra using mice were evaluated. Acute toxicity study and phytochemical screening of the extract were also determined using standard protocols. The sedative effect of the extract was evaluated using Diazepam and ketamine- induced sleep, hole board test and mouse beam walk assay, whereas the anxiolytic activity was studied using open field, elevated plus maze and elevated stair case tests.*

Results: *The intraperitonial LD_{50} of the methanol leaf extract of Ceiba pentandra was estimated to be 2150 mg/kg body weight in mice. Preliminary phytochemical screening of the extract revealed the positive reaction of saponins, flavonoids, terpenoids and tannins. The extract at doses of 300 and 600 mg/kg shortened the onset of sleep and prolonged the duration of diazepam-induced sleep. The extract at all doses tested (150,300 and 600 mg/kg) had no effect on mean onset of sleep but significantly ($p<0.05$) prolonged the duration of ketamine-induced sleep when compared with normal saline treated group. The extract at the doses of 300 and 600 mg/kg significantly ($p<0.05$) decreased the number of head dips when compared with the control group in the Hole-board test. The extract at all doses tested has no effect on the mean time spent on the beam. However, at the dose of 600 mg/kg, it significantly ($p<0.05$) increased the number of foot slips made by mice when compared with the control group. In the open field test, the extract at all doses tested (150, 300 and 600 mg/kg) significantly ($p<0.05$) decreased the number of peripheral square crossing without any effect on the number of centre square crossing. The extract had no effect on the mean number of open arm and closed arm entries, time spent in open arm and time spent in the closed arm. In the elevated staircase test, the extract significantly ($p<0.05$) reduced the number of stairs climbed and the number of rearing.*

Conclusion: *The results of this work revealed that methanol leaf extract of Ceiba pentandra contains bioactive components that possess sedative properties and hence can be used to treat insomnia in the nearest future.*

Keywords: Sedative, Anxiolytic, Insomnia, LD50, Phytochemistry

Abbreviation List

NS= Normal saline
MLCP = Methanol Leaf Extract of Ceiba pentandra
DZ = Diazepam
ANOVA = One way analysis of variance
LD50 = Medial lethal dose
SEM = Standard Error of the Mean

Background

Insomnia is characterized by difficulty in initiating and maintaining sleep or experiencing non-refreshing sleep and is marked associated with day time consequences, whereas Anxiety is a state of excessive fear accompanied by motor tension, apprehension, causing impairment of memory, intelligence and psychological function [1, 2]. Sedative-hypnotics have been used in clinical practice for the treatment of a variety of diseases related to the central nervous system which includes but not limited to acute and chronic anxiety, seizure and insomnia [3].

Insomnia is often considered to be a disorder of hyper arousal or increased somatic, cognitive and cortical activation [4]. Individuals with insomnia may experience physiologic hyper arousal in both central (cortical) and peripheral (autonomic) nervous systems. Hyper arousal in insomnia can also refer to cognitive and emotional

processes with several theories suggesting that cognitive and affective hyper arousal at bed time may contribute to both acute and chronic insomnia [5].

The use of traditional medicine in developed as well as developing countries as basis for the treatment of many ailments has been in existence for thousands of years and there is no doubt that their importance has been widely acknowledged. It is reported that 60-85% of the population in every country of the developing world has to rely on traditional or indigenous forms of medicine [6].

Plants such as *Coriandum sativum* seeds, *Ocimum gratissimum* and *Citrus aurantifolia*, *Aspilia africana*, *Vernonia amygdalena*, and Cnidoscolu sacontifolius have been scientifically validated as being effective as sedatives. In traditional medicine, plants such as *Fumerica indica*, *Azadirachta indica*, *Gelsemium sempervirens*, *Piper methysticum* & *Hypericum perforatum*, *Stachy slavandulifolia*, *Valeriana officinalis*, and *Melissa officinalis* have been reported to possess anxiolytic action as well as hypnotic effect [7-16].

Ceiba pentandra (L) (Malvaceae), known as silk cotton tree is widely used in the African traditional medicine. It is a very large, deciduous tree up to 60 m tall, with roots spreading quite horizontally, 10 m or longer, in the upper 40–80cm of the soil [17]. *Ceiba pentandra* is a tall deciduous tree supported by pronounced buttresses at the base. It can be found in various parts of moist evergreen and deciduous forests as well as in dry and gallery forests [18]. It is also normally found in the wild forest of West Africa in the tropical forest regions and in Nigeria it's popularly called "Rimi" in Hausa, "Akpu ogwu" in Igbo and "Araba" in Yoruba [19].

Methods

Animals

Swiss albino mice (17 to 25 g) obtained from Animal House of Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria were used for this study. Mice were housed and allowed to acclimatize with free access to food and water in the Animal House, of the Department of Pharmacology and Therapeutics, Bayero University, Kano and maintained under standard laboratory conditions in accordance with principles of laboratory animal care [20].

Drugs, Solvents and Equipment

Diazepam (Roche Product Ltd.), and Ketamine hydrochloride (Kwality Pharmaceuticals Pvt. Ltd.), Methanol (Sigma Chemical Ltd) and distilled water.

Collection of plant material and extract preparation

The leaves of the plant were collected from Bayero University, old site, Gwale LGA, Kano State Nigeria. It was identified and authenticated by Mallam Baha'udeen Sa'id Adam of the Herbarium Section of Plant Biology Department, Bayero University Kano by comparing with a voucher specimen number (No. BUKHAN 0471). Fresh plant materials (leaves) of *Ceiba pentandra* were dried under shade after which they were blended using mortar and pestle and sieved until a fine powder that weighed 501g was produced. The powdered plant material was macerated with 6 litres of 70% v/v methanol in a container for 3 days with occasional stirring and agitations which was filtered and the filtrate evaporated on water bath at 45°C. The dried material was stored in an airtight container. The solutions of the extract were always freshly prepared for each study by dissolving appropriate quantity required in distilled water under standard laboratory condition.

Phytochemical screening

Phytochemical screening of the two fractions was carried out using the method described by Trease and Evans [21].

Acute toxicity study

The median lethal dose (LD_{50}) of the two fractions was estimated using the method described by Lorke [22].

Sedative Activity Studies

Diazepam-induced Sleep Test on Mice

The method of Rakotonirina *et al*. was employed [23]. Twenty- four mice were divided into four groups of six mice each. The first group was treated with Normal saline 10 ml/ kg *i.p*; the second, third and fourth groups were pre-treated with 600, 300 and 150 mg/kg of the methanol leaf extract of *C. pentandra i.p*. Thirty Minutes later, mice in all the groups were treated with diazepam 2mg/kg. The criteria for sleep was considered to be loss of righting reflex and sleeping time was therefore measured as the time between disappearance and recovery of righting reflex [24].

Ketamine induced Sleep Test on Mice

The method described by Mimura *et al*. was adopted [25]. Twenty-four mice were divided into four groups of six mice each. The first group was treated with normal saline 10 ml /kg, the second, third and fourth group were pre-treated with 600, 300 and 150 mg/kg of the methanol leaf extract of *C. pentandra i.p*. Thirty minutes post treatment with the extract and normal saline, the animals were administered with ketamine (100 mg/kg) *i.p*. The time interval between ketamine administration and loss of righting reflex was considered as the onset of sleep while the time from the loss to regaining of righting reflex as the duration of sleep [26].

Hole board Test on Mice

The method described by File & Pellow, was employed [27]. Thirty mice were divided into five groups of six mice each. The first group was treated with normal saline 10 ml/kg *i.p*; the second, third and fourth groups were treated with 600, 300 and 150 mg/kg of the methanol leaf extract of *Ceiba pentandra* while the fifth group was treated with 0.25 mg/kg body weight of diazepam intraperitoneally. The test was carried out thirty minutes after various treatments. The number of head dips in 5 minutes was recorded indicating exploratory behavior. Normal saline (10 ml/kg) and diazepam (0.25 mg/kg) were used as negative and positive controls respectively.

Mouse Beam Walking Test on Mice

The method described by Stanley *et al*. was employed [28]. Thirty mice were divided into five groups of six mice each. The first group was treated with normal saline 10 ml/kg *i.p*; the second, third and fourth groups were treated with 600, 300 and 150 mg/kg of the methanol leaf extract of *Ceiba pentandra* while the fifth group was treated with 0.25 mg/kg body weight of diazepam *i.p*. The test was carried out 30 minutes after intraperitoneal treatment of the normal saline, extract and diazepam. The measurements taken were time spent on the beam (maximum of 60 seconds was allowed for each mouse on the beam), the number of foot slips (one or both hind limbs slipped from the beam) and the number of falls were recorded.

Anxiolytic Activity Studies

Open Field Test on Mice

The method described by Prut Belzung was employed [29]. The open field arena consists of 70 x 70cm wooden box of 35cm high in which the floor is divided into 16 squares (15x 15cm). Pretreatment was carried out intraperitoneally as group I received normal saline, groups II- 1V received methanol leaf extract of *C. pentandra* (600, 300 & 150 mg/kg) respectively. Group V received diazepam 0.25mg/kg body weight. Each mouse was placed at the centre square of the open field which was novel to the animal. The number of peripheral and centre squares entered by all four paws is scored for 5 minutes [30]. The arena is cleaned with 10% Ethanol solution after every test.

Elevated plus Maze Test in Mice

The method described by Hogg was adopted [31]. The apparatus consists of two open arms 30 x 5 cm and two closed arms 30 x 5 x15 cm that extend to a central platform (5 x 5 cm). Thirty mice were divided into five groups of six mice each. The first group was treated with normal saline 10 ml/kg *i.p*; the second, third and fourth groups were treated with 600, 300 and 150 mg/kg of the methanol leaf extract of *Ceiba pentandra* while the fifth group was treated with 0.25 mg/kg body weight of diazepam *i.p*. Thirty minutes post treatment, mice were individually placed on the open arm facing the centre of the maze. The number of entries and the time spent in the open and closed arms are recorded during a 5 minutes' test period. Between each test session, the maze is cleaned with a damp cotton wool containing 10% ethanol.

Elevated Staircase Test in Mice

This test was carried out according to the method described by Simiand *et al*. [32]. The staircase was made of wood and consisted of five identical steps 2.5 cm high, 10 cm wide and 7.5 cm deep. Five groups of six mice each were intraperitoneally treated as follows: group I (negative control) received normal saline 10 ml/kg body weight, groups II – 1V received methanol leaf extract of *Ceiba pentandra* while group V received diazepam 0.25mg/kg body weight. Thirty minutes after treatment, the mice were placed singly on the floor of the staircase. During a 3 minutes' period, the number of stairs climbed and the number of rearing made were recorded. A stair is considered to be climbed, when the mouse has placed all its four paws on the staircase. The box is cleaned after each test session with 10 % ethanol.

Statistical analysis

Results were expressed as Mean ± Standard Error of the Mean (SEM). Statistical analysis for difference between means were carried out using one way analysis of variance (ANOVA) followed by Dunnett's post hoc test. Values of $p < 0.05$ were considered significant.

Results

Percentage Yield of Methanol Leaf Extract of *C. pentandra*.

Extraction of 501g leaf extract of *Ceiba pentandra* with 70% methanol gave a yield of 55g (10.98% w/w).

Phytochemical Constituents of the Methanol Leaf Extract of *C pentandra*.

Preliminary phytochemical screening of methanol leaf extract of *Ceiba pentandra* revealed the presence of flavonoids, tannins, glycosides, terpenoids, phenols and saponins (Table 1).

Table 1: Phytochemical Constituents of the Methanol Leaf Extract of *Ceiba pentandra*

Constituent	Inference
Flavonoids	+
Terpenoid	+
Glycoside	+
Tannins	+
Saponins	+
Phenols	+

Key + = Present, - = Absent

Median Lethal Dose (LD50) Values of the Methanol Leaf Extract of *C. pentandra* in Mice

The intraperitonial median lethal dose (LD_{50}) value of methanol leaf extract of *C. pentandra* in mice was estimated to be 2150 mg/kg.

Effect of Methanol Leaf Extract of Ceiba pentandra on Diazepam-induced Sleep Test in Mice

The extract at doses of 600 and 300 mg/kg showed a significant decrease ($P<0.05$) in time of sleep onset and duration of sleep at all doses when compared with the normal saline group (Table 2).

Table 2: Effect of Methanol Leaf Extract of *C. pentandra* on Diazepam – induced sleep test in Mice

Treatment (mg/kg)	Mean Onset of Sleep (min)	Mean Duration of Sleep (min)
NS (10 ml/kg)	3.33 ± 0.21	27.83 ± 3.28
MLCP (150)	2.50 ± 0.22	64.17 ± 8.37*
MLCP (300)	2.33 ± 0.33*	78.50 ± 9.30*
MLCP (600)	2.17 ± 0.17*	124.50 ± 12.26**

Data presented as Mean ± SEM. *$P < 0.05$, **$P<0.01$, compared to normal saline group, using One-way ANOVA followed by Dunnett's Post hoc, n=6, NS- Normal Saline, MLCP- Methanol Leaf Extract of *Ceiba pentandra*.

Effect of Methanol Leaf Extract of *C. pentandra* on Ketamine-induced Sleep Test in Mice

The methanol leaf extract of *Ceiba pentandra* at all doses did not produce a significant ($P<0.05$) decrease in the onset of sleep when compared with the control group but there was a significant ($P<0.05$) increase in the duration of sleep at all doses of the extract when compared with the normal saline group (Table 3).

Table 3: Effect of Methanol Leaf Extract of *C. pentandra* on Ketamine-induced Sleep Test in Mice

Treatment (mg/kg)	Mean Onset of Sleep (min)	Mean Duration of Sleep (min)
NS (10 ml/kg)	3.17 ± 1.17	17.50 ± 8.87
MLCP (150)	7.33 ± 0.33	28.00 ± 1.77*
MLCP (300)	4.83 ± 0.17	30.83 ± 4.28*
MLCP (600)	2.33 ± 0.21	41.17 ± 3.29*

Data presented as Mean ± SEM. *P < 0.05, compared to normal saline group, using One-way ANOVA followed by Dunnett's Post hoc, n=6, NS- Normal Saline, MLCP- Methanol Leaf Extract of *Ceiba pentandra*.

Effect of Methanol Leaf Extract of *C. pentandra* on Hole Board Test in Mice

The extract at doses of 600 mg/kg and 300 mg/kg and the standard drug Diazepam produced significant (P<0.05) decrease in the number of head dips when compared with the normal saline group (Figure 1).

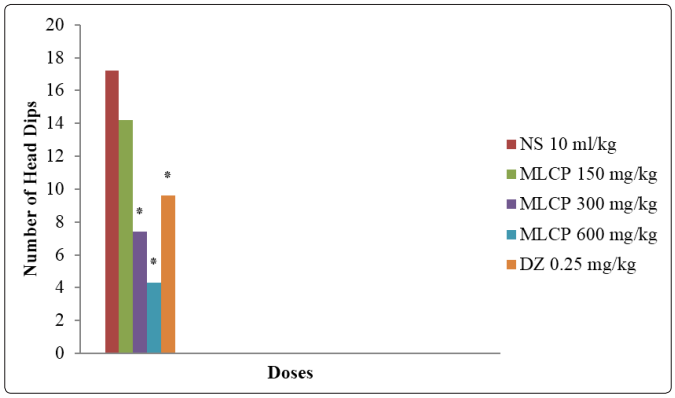

Figure 1: Effect of Methanol Leaf Extract of *Ceiba pentandra* on Hole Board Test in Mice

Data presented as Mean ± SEM. *P <0.05, compared to normal saline group using One-way ANOVA followed by Dunnett's post hoc, n=6, NS- Normal Saline, MLCP- Methanol Leaf Extract of *Ceiba pentandra*, DZ- Diazepam.

Effect of Methanol Leaf Extract of C. pentandra on Mouse Beam Walking Assay in Mice

The extract of at all doses did not significantly affect the number of times spent on the beam. However, at dose of 600mg/kg, the extract and the standard drug Diazepam, produced a significant (P<0.05) increase in the number of foot slips when compared to the normal saline group (Table 4)

Table 4: Effect of Methanol Leaf Extract of *C. pentandra* on Mouse Beam Walking Assay in Mice

Treatment (mg/kg)	Mean Time Spent on the Beam (min)	Mean Number of Foot slips
NS (10 ml/kg)	7.67 ± 1.54	0.17 ± 0.17
MLCP (150)	9.50 ± 2.43	0.17 ± 0.17
MLCP (300)	9.17 ± 2.78	1.33 ±0.21
MLCP (600)	6.00 ± 2.10	3.17 ± 1.05*
DZ (0.25)	10.27±1.64	5.17±0.48*

Data presented as Mean ± SEM. *P <0.05, compared to normal saline group using One-way ANOVA followed by Dunnett's post hoc, n=6, NS- Normal Saline, MLCP- Methanol Leaf Extract of *Ceiba pentandra*, DZ- Diazepam.

Effect of Methanol Leaf Extract of *C. pentandra* on Open Field Test in Mice

In the open field test, there was no significant difference in the number of centre square crossing for the methanol leaf extract at all doses. However, there was a significant decrease (P<0.05) in peripheral square crossing at all doses tested (Table 5).

Table 5: Effect of Methanol Leaf Extract of *C. pentandra* on Open Field Test in Mice

Treatment (mg/kg)	Mean Time Spent on the Beam (min)	Mean Number of Foot slips
NS (10 ml/kg)	50.50± 12.55	0.00 ± 0.00
MLCP (150)	13.83 ± 2.06*	0.17 ± 0.17
MLCP (300)	12.00 ± 1.79*	0.00 ± 0.00
MLCP (600)	3.83 ± 1.78*	0.50 ± 0.34
DZ (0.25)	59.83±9.39	1.00±0.52*

Data presented as Mean ± SEM. *P < 0.05, compared to normal saline group using One-way ANOVA followed by Dunnett's Post hoc test, n=6, NS- Normal saline, MLCP- Methanol Leaf Extract of *Ceiba pentandra*, DZ- Diazepam.

Effect of Methanol Leaf Extract of *C. pentandra* on Elevated plus Maze Test in Mice

There was no significant difference in the number of entries in the open arm and time spent in the open arm at all the doses of the methanol leaf extract. At all doses of the extract, there was also no significant difference in the number of entries and the time spent in the closed arm when compared with the normal saline group (Table 6).

Table 6: Effect of Methanol Leaf Extract of *C. pentandra* on Elevated plus Maze Test in Mice

Treatment in (mg/kg)	Mean Open Arms Entry	Mean Duration in Open Arms	Mean Closed Arms Entry	Mean Duration in Closed Arms
NS (10 ml/kg)	1.00 ± 0.52	8.67 ±4.01	7.67±1.61	232.33±12.87
MLCP (150)	0.17 ± 0.17	6.17 ±4.35	2.67±0.61	212.83 ±25.71
MLCP (300)	0.33 ± 0.21	8.17 ±8.17	1.67±0.49	248.17 ±24.73
MLCP (600)	0.50 ± 0.22	7.00 ±5.26	2.00±0.82	251.83 ±16.83
DZ (0.25)	2.17 ± 1.40*	16.00 ±7.58	10.67±2.32	188.83 ±26.77

Data presented as Mean ± SEM. *P <0.05, compared to normal saline group using One-way ANOVA followed by Dunnett's post hoc, n=6, NS- Normal Saline, MLCP- Methanol Leaf Extract of *Ceiba pentandra*, DZ- Diazepam.

Effect of Methanol Leaf Extract of *C. pentandra* on Elevated Staircase Test in Mice

The extract at all doses produced a significant (P<0.05) decrease in the number of stairs climbed and in the number of rearing when compared with the normal saline treated group (Table 7).

Table 7: Effect of Methanol Leaf Extract of *C. pentandra* on Elevated Staircase Test in Mice

Treatment (mg/kg)	Mean Number of Stairs Climbed	Mean Number of Rearing
NS (10 ml/kg)	22.67 ± 5.02	13.83±3.15
MLCP (150)	11.33 ± 2.22*	2.67±0.49*
MLCP (300)	10.00 ± 1.88*	2.50±1.59*
MLCP (600)	6.00 ± 2.11*	0.67±0.49*
DZ (0.25)	55.50 ± 11.74	14.50±3.04

Data presented as Mean ± SEM. *$P < 0.05$, compared to normal saline group using One-way ANOVA followed by Dunnett's post hoc, n=6, NS- Normal Saline, MLCP- Methanol Leaf Extract of *Ceiba pentandra*, DZ- Diazepam.

Discussion

Preliminary phytochemical screening of the methanol leaf extract of *Ceiba pentandra* gave a positive reaction of flavonoids, terpenoids, glycosides, tannins, saponins, and phenols, which is similar to the phytochemical components observed by Enechi *et al*. (19) using same extract. However Sule *et al* [33]. observed the presence of additional secondary metabolites such as flavonoids, saponins, tannins and alkaloids in the methanol stem bark extract of *Ceiba pentandra*.

These bioactive compounds (Flavonoids, Phenols and Saponins) are known to exhibit medicinal activity as well as physiological activity. According to Akindele & Adeyemi, the anxiolytic and sedative activities of *Byrsocarpus coccineus* were possibly due to the presence of flavonoids and terpenoids. Also, the sedative activity of the methanol root bark extract of *Securinega virosa* may be due to the presence of saponins and flavonoids. It has also been reported that saponins show a potent sedative activity .Therefore, flavonoids, saponins and terpenoids may be responsible for the sedative activity observed in this study [34-35].

Median lethal dose is one of the indices of acute toxicity studies [36]. LD50 is the dose that will kill fifty percent of a population. In this experiment, the intraperitoneal median lethal dose of methanol leaf extract of *Ceiba pentandra* in mice was estimated to be 2150 mg/kg which is considered to be relatively toxic according to Lorke.

The extract reduced the onset of sleep induced by diazepam and increased the duration of sleep, suggesting that the extract seems to possess sleep inducing properties. Sedative hypnotic agents act to increase GABA- mediated synaptic inhibition either by directly activating GABA receptors or by enhancing the action of GABA on GABAA receptors. The ability of the extract to potentiate the sedative property of diazepam suggests that it may possibly act by interacting with GABA- mediated synaptic transmission.

The extract at all doses did not produce a significant effect in the onset of sleep induced by ketamine but significantly ($P<0.05$) increased the duration of sleep suggesting that the extract may be beneficial in the maintenance of sleep rather than facilitating sleep induction by possibly blocking NMDA receptors [37].

The extract and standard drug Diazepam significantly ($P<0.05$) produced a decrease in the exploratory behavior pattern as shown in the reduction of head dip result counts. According to File & Pellow the hole board experiment is a measure of exploratory behavior in animals. A decrease in this parameter reveals a sedative behavior. This effects of the extract on exploratory behavior further strengthens our speculations that it may contain bioactive principles that are active in nature.

In the mouse beam walking assay test, the extract significantly ($P<0.05$) increased the number of footslips made by the mice. The number of footslips has been found to be a sensitive measure of determining benzodiazepine- induced motor coordination deficits and a better predictor of doses producing sedation clinically [38].

In the open field test, the methanol leaf extract of *Ceiba pentandra* did not produce a significant difference in the number of centre square crossing, but showed a significant ($P<0.05$) decrease in the number of peripheral square crossing when compared to the normal saline group. Peripheral square crossing is an index of locomotion, and its decrease indicates a CNS inhibitory activity suggesting a sedative effect.

The extract did not produce a significant decrease in the number of entries in open arm and time spent in open arm in the elevated plus maze test and there was no significant difference in the number of entries and time spent in the closed arm suggesting that there is no manifestation of fear and anxiety [39].

The methanol extract of *Ceiba pentandra* decreased significantly ($P<0.05$) the number of rearing and the number of steps climbed by the mice in the elevated staircase test, suggesting no anxiolytic effect.

Conclusion

Results obtained from this study revealed that the methanol leaf extract of *Ceiba pentandra* contain bioactive substances that possess sedative activity and hence can be used to treat insomnia in the nearest future.

Authors' Contribution

In this work, the use of Ceiba pentandra in our localities for the treatment of insomnia has been scientifically justified and the results could serve as a baseline data for carrying out other researches.

Conflict of interest declaration

Authors declare no conflict of interest

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