EFFECTS OF AQUEOUS LEAF EXTRACTS OF *Murraya koenigii* ON LEARNING AND MEMORY IN MICE

Abstract

Curry (*Muraya koenigii*) leaf is an essential leafy spice used widely in cuisine for its distinct flavor and for other medicinal purposes: analgesic, antidysenteric, antioxidant and in regulating fertility. The Morris water maze was used to study the effects of aqueous extracts of *M. koenigii* (curry) leaf on learning and memory. Aqueous leaf extracts of *M. koenigii* (80mg/kg, p.o.) was administered to 7 CD1 strain of mice (18-28g body weight) while the control group received 0.1ml/10kg body weight of distilled water (orally) for 10 days before behavior was assessed. All mice were tested in the Morris water maze for 8 days: at 4 trials per day and 60 seconds per trial. Day 1-3 were for acquisition training, day 4-6 reversal training, day 7, the probe trail and day 8 visible platform task. Results indicate that swim latency were not significantly different between the groups during acquisition and reversal training. The retention quadrant duration was significantly higher for the *M. koenigi*-treated mice compare to the control ($P<0.05$). The mice treated with *M. koenigi* showed a negative weight gain, indicating weight loss ($p< 0.05$). Therefore the aqueous extracts of *M. koenigi* improved visuospatial memory in the mice and decreased body weight.

Keywords: *Muraya koenigi*, memory, acquisition and reversal training, body weight

1.0 Introduction

From time immemorial man has use plants for food and for medicinal purposes as prevention of infections and curing of diseases. Man had relied so much on these plants that even in modern medicine, these herbs are used in modernized form for various medicinal purposes. One of these plants that has come to have so much impact in the lives of human in recent decades is *Murraya koenigii* (curry plant).

Murraya koenigii is a minor member of *Rutaceae* family and a distant relative of citrus fruits that originated from southern Asia. The leaf is frequently used as flavor enhancers in south Indian cooked food and also used as cooking items in food recipe. The leaves are highly aromatic and are used as herbs with the major constituents responsible for the aroma and flavor being reported as caryophellene, pinene, sabinene, cadinol and cadinene (Nigam *et al*., 1961).

The leaves are food ingredients as well as medicinal ingredients used to relieve nausea, indigestion, vomiting, and it's eaten for cure of diarrhea and dysentery (Ghani, 2003). The leaves are stimulant and astringent and are used in the treatment of coughs and hysteria (Ghani, 2003). The essential oil (tannins) found in the leaves shows significant anti- inflammatory and analgesic activities (Dash *et al*, 2004). Sawanjaroen reported that the plant showed anti-amoebic activities (Sawanjaroen, 2006) and other studies shows that the leaves and other tissues have both stimulant and astringent properties and are used to treat wounds, joints pains, body ache (Parrota, 2001) and also as an abortive (Xiao & Wang, 1991).

The curative power of this plant is in its ability to improve the functioning of the stomach and the small intestine and probably to promote their actions. Paste of the leaves with lime juice and

honey is a time tested medicine in the treatment of hyperemesis gravidarum (severe form of nausea and vomiting in pregnancy (Goswami, 2002).

The stems and the roots have been used for the treatment of certain dermatological diseases such as skin irritation (rashes) and poisonous bites. The fruits are used in Burma for improving digestive system by initiating peristaltic wave. The leaf extract is used as hair wash to remove dandruff (Perry 1980), and as tonic and stomachic.

The extraction of the seed was found to possess antifungal and antimicrobial property (Gautam *et al*., 1974), but recent studies on *Murraya koneigii* includes reports on its hypoglycemic activities (Yadav, 2002), anti- asthmatic effect (Walde, 2004), anti-oxidant activity (Tachibana, 2001), anti- fungal activity (Kishore *et al* , 1982), anxiolytic effect (Bisong *et al*, 2017) , and as fertility enhancer (Mehrota, 2005) etc.

In an analysis, the quality of curry leaf as herbal tonic, G. K. Nair (2001) of the University of Agricultural Science (UAS) Dharward, reported that the leaves are packed with minerals, vitamins A and B and are rich sources of carbohydrates, protein and alkaloids etc. He also reported its stem bark as acrid, cooling, anaethematic and analgesic properties and its use in managing piles, allay heat of the body and in leucoderma and blood disorder.

It is believed that the edible portion of the fruits contains good distribution of minerals like phosphorus, calcium, potassium, magnesium, iron and protein. It contains an alkaloid known as *murraya acinine* (Charkaborty, 1974) which according to cardiologist is a gastro- intestinal motility regulator, and prevents eructation and bloating of the abdomen. The roots of this plant have medicinal property that relief pains associated with kidney disorder.

However, despites the many studies that have been elucidated, there is little research on the effect of *Murraya koenigii* on learning and memory. Therefore it is the aim of this study to explore the effects of aqueous leaves extracts of *Murraya koenigii* on learning and memory.

2. Materials and methods

2.1 Preparation/ Administration of Aqueous leaf-extracts of *Murraya koenigii*

Fresh leaves of *M. koenigii* (curry leaf) were collected and dried in an Astell Hearsan oven (model no. P.B.S 000, England) at a temperature range of 40c -50c. The dried leaves were ground into powdered form weighing 126g. The powdered form of the leaves was then soaked in 1100mls of deionized water and allowed to stand for 15 hours. This was then filtered using chase material. The filtrate was further filtered using Whatmann size 1 filter paper. The filtrate was then transferred into the Astell hearsan Oven set at 40-50 c to evaporate to complete dryness yielding 25g of extract resulting in about 20% yield. The dried extract was reconstituted in normal saline ad administered orally at the dose of 80 mg/kg body weight.

2.2 Experimental Animals

The animals used for the study were 14 healthy male CD1 strain of mice weighing between 18 - 28g. Animal care was as described by Bisong *et al*, (2019) and followed extant laws. The mice were exposed to a 12/12 light /dark cycle while being divided into groups. Group 1 served as

control and was administered 0.1ml/10g body weight of distilled water while group 2 served as test animals which were administered 80mg/kg of aqueous extract of *M. koenigii* leaves. This administration was done orally for 10 days before behavioral assessment were carried out.

2.3 Experimental protocol

The Morris water maze modified for mice as used by Bisong *et al* (2016) was used and the pool was divided into four quadrants; Northeast, Northwest, Southeast and Southwest. It is constructed out of a circular polypropylene pool of round container that measures 172.5cm and diameter 53cm. The water was allowed to sit over night to attain room temperature. The water was made adding more water to submerge an escape platform by approximately 1cm in one of the quadrants. The test consisted 3 days of acquisition training, 3 days of reversal training (each day consisting of 3 trials with a hidden platform 2cm below water level), a day of probe trial (single trial) and a day of visible platform task.

3 Results

3.1 Comparison between learning curves for mice administered with crude aqueous extract of *M. koenigii* leaves (80mg/kg, p.o) and their control during acquisition and reversal training.

The swim latencies for the mice administered with crude aqueous extract of *M. koenigii* leaves (80mg/kg, i.p) were not different during acquisition training. The swim latency for the *M. koenigii* group were 10.5 ± 42.18s, 7.9 ± 1.37s and 7.69 ± 2.06s for day 1, 2 and 3 of acquisition respectively while the control were 12.11± 2. 18 s; 6.57 ± 0. 842 s and 6.79 ± 1.39 s. The swim latency for the mice administered with crude aqueous extracts of *M. koenigii* leaves were also not different from the control during reversal training. The swim latencies for the group of mice administered with the extracts were 9.75 ±2.26 s; 8.54 ± 2.47 s and 6.19 ± 1. 29 s for day 1, 2 and 3 of reversal training respectively while the control were 10.04 ± 2.67 s ; 9.75 ± 2. 26 s and 5.39 ± 0.86 s respectively.

3.2 Comparison between quadrant durations for mice administered crude aqueous extracts of *M. koenigii* (80mg/kg, p.o.) and their control during probe trail in the Morris water Maze

The hidden escape platform was located in the south-west (SW) quadrant during reversal training. The quadrant duration for the *M. koenigii* treated group of mice was significantly higher compared to control at 17.18 ± 1.62 s while that for the control group was 10. 99± 1.07s ($p<0.01$).

Day 8 was the visible platform task and the escape platform was made visible and animals allowed to explore and mount it for escape. The swim latencies during the visible platform task did not differ between the *Murraya koenigii* treated mice with 4. 57 ± 0.72 s and their control was 5.57 ± 0.71 s.

3.3 The Effect of administration of crude aqueous extract of *Murraya koenigii* on body weight change.

Daily weight changes were measured during a ten day course of intraperitoneal administration of crude aqueous extract of *M. koenigii* leaves to mice. The weight change for the *M. koenigii* treated mice ranged from -0.36 ± 0.13g to -1.78 ± 0.25g, while that for the control group ranged from -0.08 ± 0.22g to -0.47 ± 0.28g. This is shown in figure 5 below.

Figure 6 shows the mean final body weight change. The chart showed that the change in body weight in the *M. koenigii* treated group of mice was greater than that for their control ($p < 0.001$). The mean final body weight change in the *M. koenigii* treated group which was -1.78 ± 0.25g was more negative compared to that for control mice which was -0.47 ± 0.28g ($p < 0.001$).

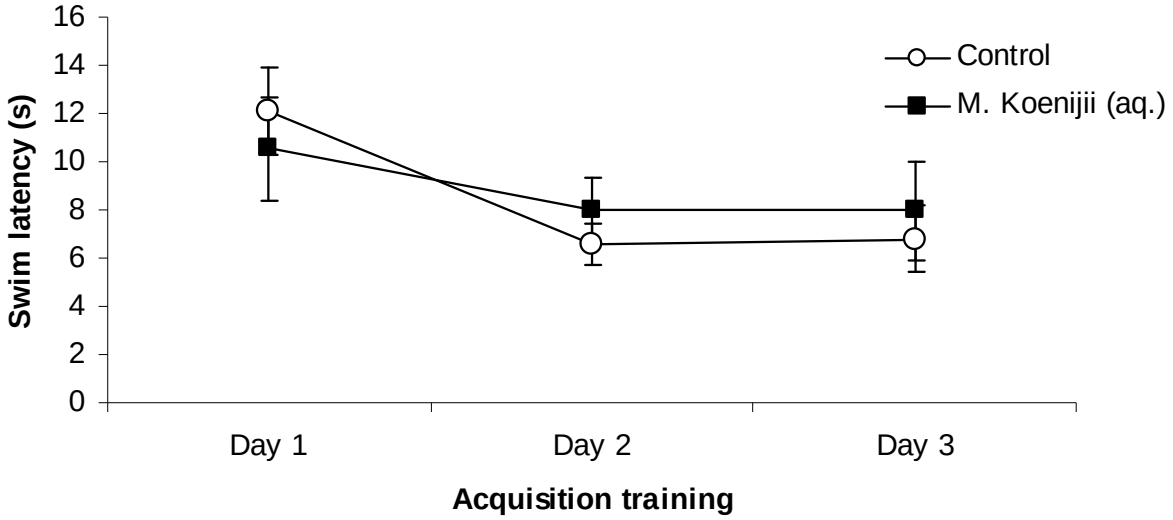
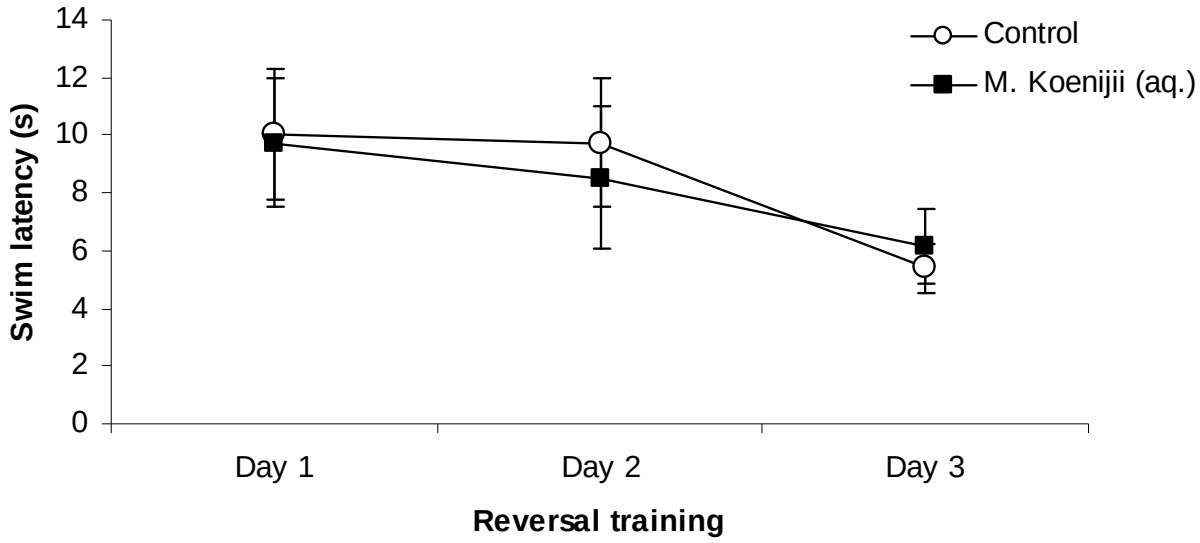


Fig 1: Comparison of learning curves for showing swim latencies for mice administered crude aqueous extract of *Murraya koenigii* leaves (80mg/kg), and their control during the acquisition training in the Morris water maze.



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138 **Fig 2: Comparison of learning curves for showing swim latencies for mice administered**
139 **crude aqueous extract of *Murraya koenigii* leaves (80mg/kg), and their control during the**
140 **reversal training in the Morris water maze.**

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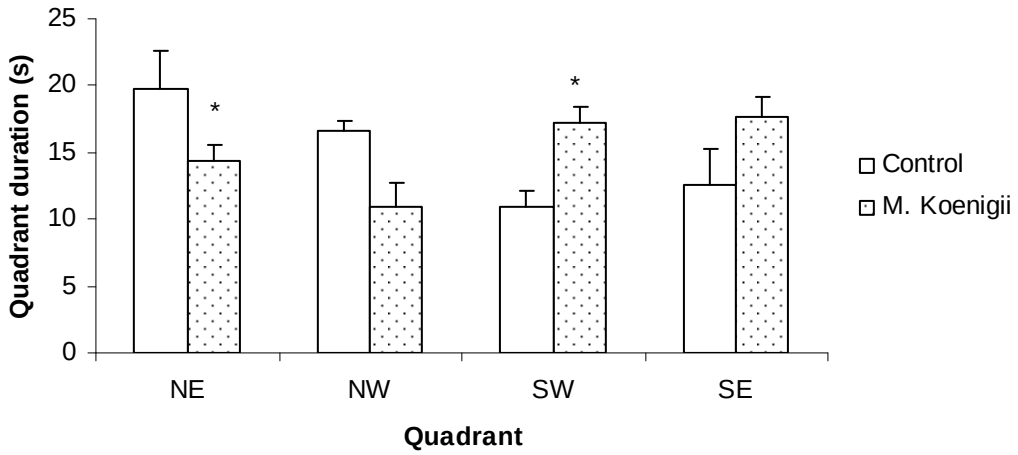

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143 * - significant at p< 0.05 compared to control.

144 **Fig 3: Comparison between quadrant duration for mice administrated crude aqueous**
145 **extract of *Murraya koenigii* leaves and their control during the probe trial in the Morris**
146 **water maze.**

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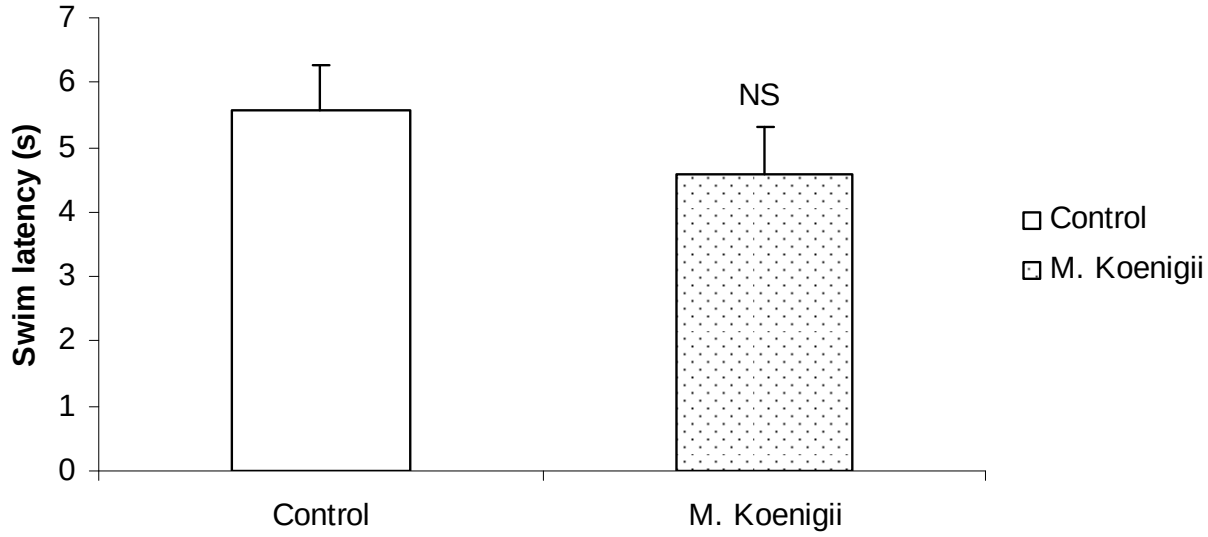

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149 NS – Not significant compared to control.

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151 **Fig 4: Comparison between quadrant duration for mice administrated crude aqueous**
152 **extract of *Murraya koenigii* leaves and their control during the probe trial in the Morris**
153 **water maze.**

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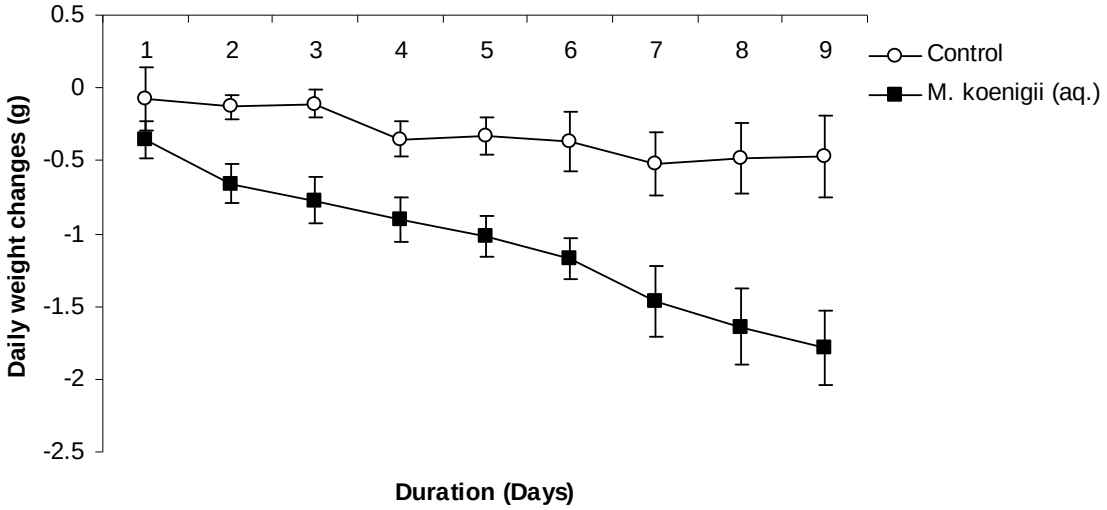
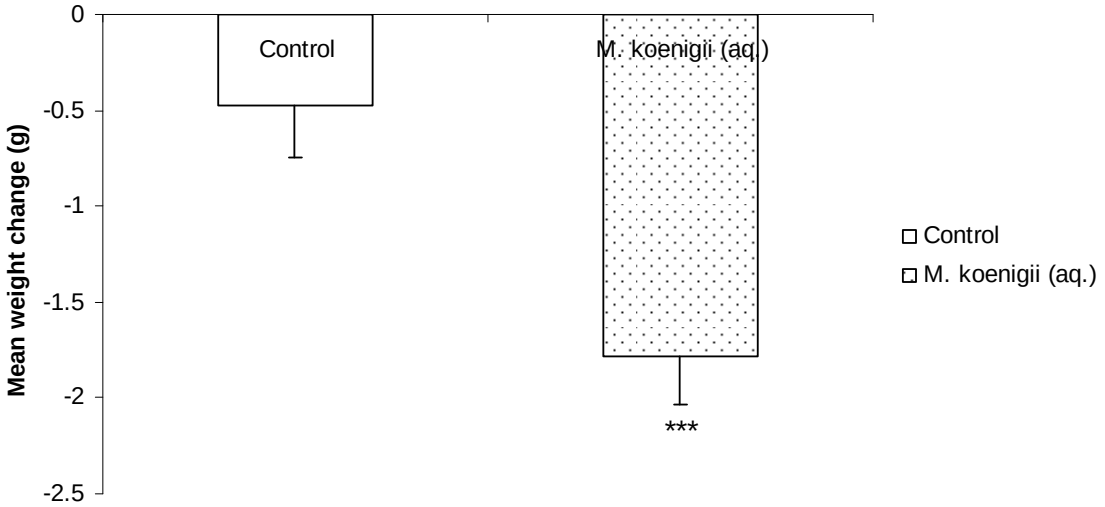


Fig 5: Body weight changes following intraperitoneal administration of crude aqueous extract of *Murraya koenigii* leaves to mice compared to their control.



*** - significant at p< 0.001 compared to control

Fig 6: Comparison between mean final body weight changes for mice administered crude aqueous extract of *Murraya koenigii* leaves (80mg/kg, i.p.) and their control.

4.1 Discussion

The Morris water maze has been used as a test for spatial learning in rodents (Morris, 1981). It is one of the most frequently used experimental paradigms to assess the effect of brain lesion and to evaluate the properties of cognitive enhancers (Morris, 1984). The Morris water maze has also been used extensively to study strain difference in spatial learning in mice (Chapilon *et al*., 2000).

In this study, the spatial learning was first employed by providing an invisible platform. The result obtained from the test showed that during the acquisition training, the swim latency did not differ between mice administered aqueous extract of *Murraya koenigii* and the controls. Both the control and the *Murraya koenigii* tested group showed a good learning curve with the swim latencies decreasing over the period of acquisition training. Thus, the control animals and the test group spent about equal time locating the hidden escape platform meaning that they learn equally well.

During the reversal training, a similar trend in the result also occurred. The swim latency did not differ between the control mice and the mice administered aqueous extract of *M. koneigii* leaf. The learning curve was consistent and both groups showed a good learning curve with a decrease in swim latency over the training period. Since lower swim latency means better learning process, this implies that the test and the control had a good performance in the Morris water maze during reversal training as well as acquisition. The implication here is that both mice were able to learn the position of the platform equally.

The south–west (SW) quadrant or Retention quadrant is the quadrant that had the hidden escape platform during the reversal training. The south – west quadrant duration was significantly higher for the *Murraya koenigii* treated group compared to control. This means that mice administered aqueous extract of *Murraya koenigii* spent more time trying to locate the hidden platform in the SW quadrant. This also implies that there was memory of the location of the platform.

However, the quadrant duration for the North East (NE) quadrant which had the platform during acquisition training was lower for the *Murraya koenigii* treated group compared to control. This implied that the control remembered acquisition quadrant better than the *M. koenigii* treated mice. This result also buttressed the implication of increased retention quadrant in the *M. koenigi* treated group of mice.

The visible platform task is used for assessing place learning and also used to assess abnormalities in the visual ability of the animals. Thus, poor platform task performance will mean poor visual ability or poor place learning ability.

The swim latencies obtained for both control and *M. koenigii* treated mice did not show any difference. This implies that both group of animals had no visual impairment and could have had good place learning ability.

Although not shown in this result, the food intake did not differ between the groups. However, the body weight of the mice in the *M. koenigi* treated group decreased showing a weight loss. This is possibly a reason why the animals were smarter in activity generally.

4.2 Conclusion

Administration of crude aqueous leaf extracts of *M. koenigii* (80mg/kg) improved memory in the Morris water maze test and decreased body weight.

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