

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

## Abstract

Curry (*Murraya 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 b.w.) while the control group received 0.1ml/10kg body weight of distilled water (p.o.) for 10 days before behavior was assessed. All mice were tested 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. koenigii*-treated mice compare to the control (P<0.05). The mice treated with *M. koenigii* showed a negative weight gain, indicating weight loss (p< 0.05). Therefore the aqueous extracts of *M. koenigii* improved visuospatial memory in the mice and decreased body weight.

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**Keywords:** *Murraya koenigii*, 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 *mMurraya koenigii* (curry plant).

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*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 relieved 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 (sSawanjaroen, 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, 2004).

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

46 The extraction of the seed was found to possess antifungal and antimicrobial property (Gautam *et*  
47 *al.*, 1974), but recent studies on *mMurraya koenigii* includes reports on its hypoglycemic  
48 activities (Yadav, 2002), anti- asthmatic effect (Walde, 2004), anti-oxidant activity (Tachibana,  
49 2001), anti- fungal activity (Kishore *et al* , 1982), anxiolytic effect (Bisong *et al*, 2017) , and as  
50 fertility enhancer (Mehrota, 2005) etc.

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

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

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

64

## 65 2. Materials and methods

### 66 2.1 Preparation/ Administration of Aqueous leaf-extracts of *Murraya koenigii*

67 Fresh leaves of *M. koenigii* (curry leaf) were collected and dried in an Astell Hearsan oven  
68 (model no. P.B.S 000, England) at a temperature range of 40c -50c. The dried leaves were  
69 ground into powdered form weighing 126g. The powdered form of the leaves was then soaked in  
70 1100mls of deionized water and allowed to stand for 15 hours. This was then filtered using chase  
71 material. The filtrate was further filtered using Whatmann size 1 filter paper. The filtrate was  
72 then transferred into the Astell hearsan Oven set at 40-50 c to evaporate to complete dryness  
73 yielding 25g of extract resulting in about 20% yield. The dried extract was reconstituted in  
74 normal saline ad administered orally at the dose of 80 mg/kg body weight.

75

### 76 2.2 Experimental Animals

77 The animals used for the study were 14 healthy male CD1 strain of mice weighing between 18 -  
78 28g. Animal care was as as described by Bisong *et al*, (2019) and followed extant laws. The  
79 mice were exposed to a 12/12 light /dark cycle while being divided into groups. Group 1 served  
80 as control and was administered 0.1ml/10g body weight of distilled water while group 2 served

81 | as test [animals which](#) were administered 80mg/kg of aqueous extract of *M. koenigii* leaves. This  
82 | administration was done orally for 10 days before behavioral assessment were carried out.

### 83 | **2.3 Experimental protocol**

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

92

## 93 | **3 Results**

### 94 | **3.1 Comparison between learning curves for mice administered [with](#) crude aqueous extract 95 | of *M. koenigii* leaves (80mg/kg, p.o) and their control during acquisition and reversal 96 | training.**

97 | The swim latencies for the mice administered [with](#) crude aqueous extract of *M. koenigii* leaves  
98 | (80mg/kg, i.p) were not different during acquisition training. The swim latency for the *M.*  
99 | *koenigii* group were  $10.5 \pm 42.18s$ ,  $7.9 \pm 1.37s$  and  $7.69 \pm 2.06s$  for day 1, 2 and 3 of acquisition  
100 | respectively while the control were  $12.11 \pm 2.18s$ ;  $6.57 \pm 0.842s$  and  $6.79 \pm 1.39s$ . The swim  
101 | latency for the mice administered [with](#) crude aqueous extracts of *M. koenigii* leaves were also not  
102 | different from the control during reversal training. The swim latencies for the group of mice  
103 | administered [with](#) the extracts were  $9.75 \pm 2.26s$ ;  $8.54 \pm 2.47s$  and  $6.19 \pm 1.29s$  for day 1, 2  
104 | and 3 of reversal training respectively while the control were  $10.04 \pm 2.67s$ ;  $9.75 \pm 2.26s$  and  
105 |  $5.39 \pm 0.86s$  respectively.

106

### 107 | **3.2 Comparison between quadrant durations for mice administered crude aqueous extracts 108 | of *M. koenigii* (80mg/kg, p.o.) and their control during probe trail in the Morris water 109 | Maze**

110 | The hidden escape platform was located in the south-west (SW) quadrant during reversal  
111 | training. The quadrant duration for the *M. koenigii* treated group of mice was significantly higher  
112 | compared to control at  $17.18 \pm 1.62s$  while that for the control group was  $10.99 \pm 1.07s$   
113 | ( $p < 0.01$ ).

114 | Day 8 was the visible platform task and the escape platform was made visible and animals  
115 | allowed to explore and mount it for escape. The swim latencies during the visible platform task  
116 | did not differ between the *Murraya koenigii* treated mice with  $4.57 \pm 0.72s$  and their control  
117 | was  $5.57 \pm 0.71s$ .

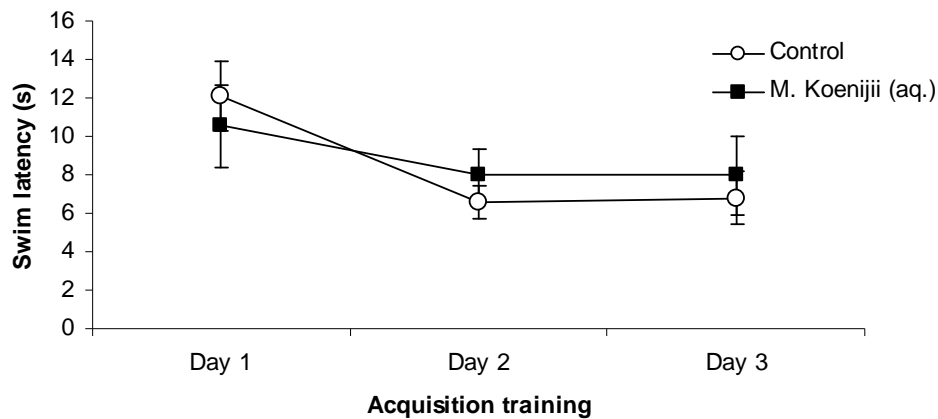
### 118 | **3.3 The Effect of administration of crude aqueous extract of *Murraya koenigii* on body 119 | weight change.**

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

124 Figure 6 shows the mean final body weight change. The chart showed that the change in  
125 body weight in the *M. koenigii* treated group of mice was greater than that for their control ( $p <$   
126  $0.001$ ). The mean final body weight change in the *M. koenigii* treated group which was  $-1.78 \pm$   
127  $0.25\text{g}$  was more negative compared to that for control mice which was  $-0.47 \pm 0.28\text{g}$  ( $p < 0.001$ ).

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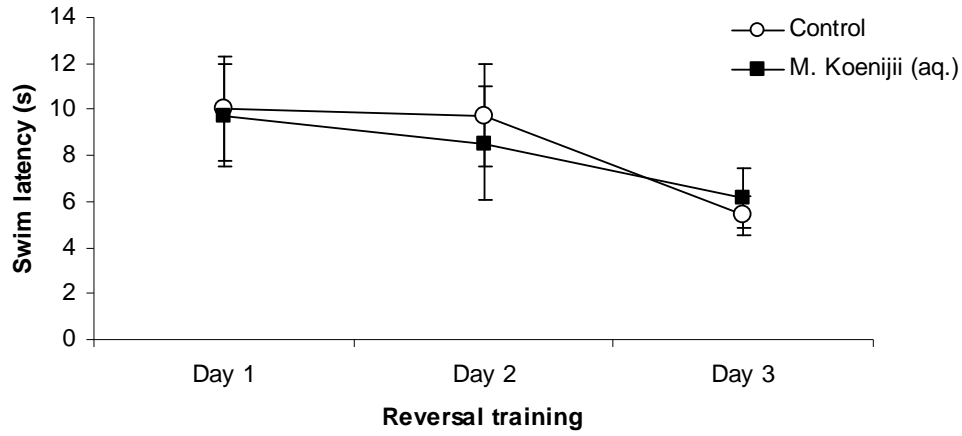


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132 **Fig 1: Comparison of learning curves for showing swim latencies for mice administered**  
133 **crude aqueous extract of *Murraya koenigii* leaves (80mg/kg), and their control during the**  
134 **acquisition training in the Morris water maze.**

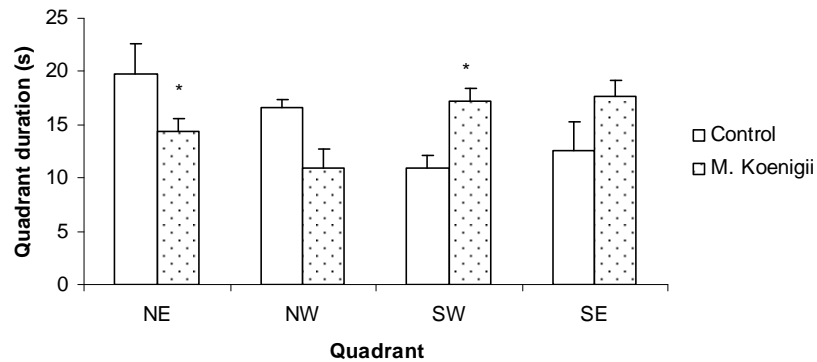
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137 **Fig 2: Comparison of learning curves for showing swim latencies for mice administered**  
138 **crude aqueous extract of *Murraya koenigii* leaves (80mg/kg), and their control during the**  
139 **reversal training in the Morris water maze.**

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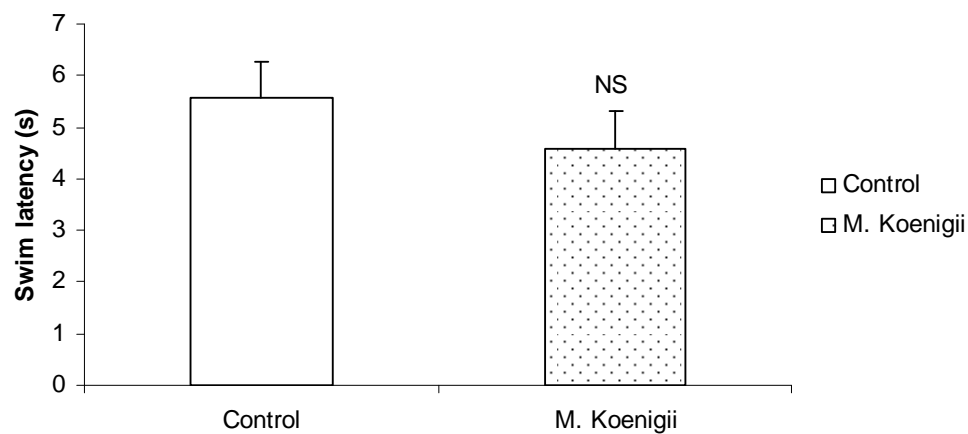


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142 \* - significant at  $p < 0.05$  compared to control.

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

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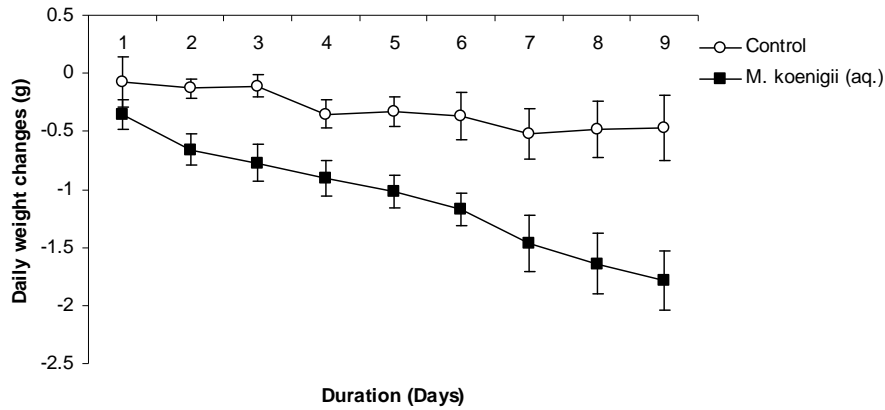
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148 NS – Not significant compared to control.

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

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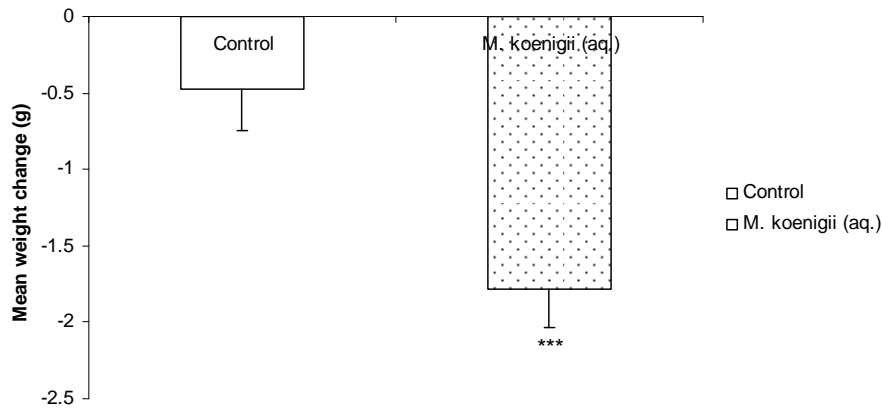


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156 Fig 5: Body weight changes following intraperitoneal administration of crude aqueous extract of  
 157 *Murraya koenigii* leaves to mice compared to their control.

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159

160 \*\*\* - significant at  $p < 0.001$  compared to control

161

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

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165

#### 166 4.1 Discussion

167 | The Morris water maze has been used as a test for spatial learning in rodents (Morris, 1981). It is  
168 | one of the most frequently used experimental paradigms to assess the effect of brain lesion and to  
169 | evaluate the properties of cognitive enhancers (Morris, 1984). The Morris water maze has also  
170 | been used extensively to study strain difference in spatial learning in mice (Chapillon *et al.*,  
171 | 2000).

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

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

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

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

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

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

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

206

Comment [U1]: How was this measured?



207 **4.2 Conclusion**

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

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