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Effect of ethanolic extract of *Carpolobia lutea* G. Don (polygalaceae) root on learning and memory in CD1 mice

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Summary: *Carpolobia lutea*, commonly called cattle stick or poor man's candle, is used by traditional herbalists in eastern Nigeria to treat 'madness'. It has a reported analgesic and anti-nociceptive effect. The effect of its ethanolic root extract on learning and memory was investigated. Thirty mice were divided into three groups of ten each. One group of mice served as the control and was given normal saline (p.o.) while the other two groups were given acute low dose (1500mg/kg, p.o.) and high dose (2500mg/kg, p.o.) (LD_{50} 3338.83mg/kg). The effect of the extract on cognitive memory was investigated using the Novel Object recognition task (NORT) while the effect on visuospatial learning and memory was studied using the Morris Water maze (MWM). The results obtained in the NORT show that the index of habituation was significantly lower following acute treatment with a low dose of *C. lutea* extract compared to control. However, the index of habituation did not differ following treatment with a high dose of *C. lutea* compared to control but it was higher compared to the low dose. Following treatment with a low dose of the extract, the index of discrimination was significantly higher compared to control. The index of discrimination in the high dose treatment group did not differ from control, but it was lower compared to the low dose treatment. This indicated that there was improved cognitive memory only in the low dose treatment group. In the MWM there was no significant difference in swim latency during Acquisition and Reversal training. There also was no significant difference in quadrant duration during probe trial. The swim latency during the visible platform test showed that all mice used had good visual acuity. Therefore, the ethanolic extract of *C. lutea* root enhanced cognitive memory. However it did not affect visuospatial learning and memory.

Keywords*: Carpolobia lutea* root, Cognitive Memory, Visuospatial memory, Mice

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INTRODUCTION

Many plants/herbs or natural products have been found to enhance learning and memory. Some of these natural products enhance memory by their inherent anti-oxidant ability (Abdullah, 2011; Zhang *et al*, 2012; Essa *et al*, 2012). *Carpolobia lutea* has since been used traditionally for the treatment of various ailments such as sterility, worm infestation, 'madness', dermal infections, veneral diseases and malaria (Burkill, 1985; Mitaine-offer *et al*, 2002). It is known in the southern regions of Nigeria by different names. The Ibibios call it *Ndiyan*, the Efiks call it Ikpafum, the Igbos call it Agba or Angalagala, the Yorubas call it Egbo oshunshun or Egbo orere, the Eket people of Akwa Ibom state call it Abekpok ibuhu. *C. lutea* is a shrub (a small tree) found in West and Central Tropical Africa that grows up to 5m (approximately 15ft) in height (Hutchinson & Dalziel, 1954). Its powdered bark is taken as snuff for headache and to prevent sleep due to fatigue (Nwidu & Nwafor, 2009). *C. lutea* is regarded traditionally as a remedy for a host of ailments.

A phytochemical analysis by Ettebong and Nwafor (2009) revealed that it contains saponins, simple sugars, terpenes and cardiac glycosides in large quantity and some flavonoids and anthraquinones. Ethyl acetate leaf extract of *C. lutea* has also been reported to contain cinnamic and coumaric acid derivatives (Nwidu *et al*, 2011).

Research on the extracts of C. lutea has shown that it has many useful effects. *C. lutea* extracts have been reported to have anti-malarial activity (Okokon *et al* 2011), and anti-plasmodial activity against chloroquine resistant and chloroquine sensitive strains of Plasmodium falciparum in in-vitro studies (Bero *et al*, 2011). The leaf extract of *C. lutea* has protective action on cells of gastric mucosa via prostaglandin mobilization, inhibition of lipoxygenase pathway and anti-cholinergic effect. (Nwidu & Nwafor, 2009). Its ethanolic leaf extract inhibits intestinal transit time, castor oil-induced diarrhea and fluid accumulation. The crude extract of *C. lutea* inhibits indomethacin and ethanol-induced ulceration (Nwafor & Bassey, 2007). It has been

reported to be useful in the management of acute pain due to its peripheral analgesic and anti-nociceptive activity (Jackson *et al*, 2011; Nwidu *et al*, 2011). *C. lutea* root extract has anti-microbial activity (Idowu *et al*, 2005). It also has anti-bacterial, anti-inflammatory and anti-arthritic properties due to the presence of Saponins and flavonoids (Ettebong & Nwafor, 2009).

Studies and research into the effect of *C. lutea* has, so far, focused on the Gastrointestinal system. Little work has been done on the study of its effect on the Nervous system. The focus of this research is to contribute in reducing the dearth of knowledge in this area. Therefore, this study was aimed at investigating the effects of ethanolic extracts of *C. lutea* root on cognitive and visuo-spatial learning and memory and to determine whether the effect (if any) is dose – dependent.

MATERIALS AND METHODS

Plant Extract Preparation

Fresh roots of *C. lutea* were obtained from Afikpo North local government area of Ebonyi state and identified at the department of Botany, University of Calabar (Herbarium number 513). The roots were sliced into tiny chunks before oven drying at 40^{o}C. The dried roots were then grinded to fine powder using a manual blender. The powdered root was then subjected to maceration (cold extraction) in an extraction jar using ethanol for 72 hours. The crude extract was then filtered using Whatmann No. 1 filter paper. The filtrate was evaporated using a hot plate at 40^{0}C. The pasty concentrate was stored in a refrigerator at 4^{o}C until required for use.

Experimental animals

Thirty Swiss white albino (CD1) mice were randomly divided into three groups of ten mice each. The first group served as the control and so received normal (0.9%) saline orally. The other two groups were treated with a low dose (1500mg/kg, p.o.), and a high dose (2500mg/kg, p.o.) of the extract respectively based on a pre-determined median lethal dose (LD_{50} 3338.83mg/kg) by Jackson *et al* (2011). All animals had access to feed and water *ad libitum*.

Evaluation of learning and memory

The Novel Object Recogniton Task:

The Novel object recognition task (NORT) modified by Brown *et al* (1999) was used to test cognitive memory in a 24hrs inter trial interval. The NORT assesses a mouse's ability to recognize a familiar object over a variable length of time; this ability has been coined recognition memory. The NORT relies upon a mouse's intrinsic exploratory drive to investigate novel objects. The experimental procedure also lacks stress components, such as forced swimming or food deprivation. In mice models the amount of interaction with the novel object is assessed. Recognition memory is comprised of both familiarity detection and recollection (Aggleton & Brown, 2006; Fortin, Wright & Eichenbaum, 2004). These functions are primarily localized within the medial temporal lobe (MTL) (Bachevalier *et al*., 2005; Brown & Aggleton, 2001).

The Morris Water Maze:

The Morris water maze (MWM) modified for mice by Paylor *et al* (1996) was used to test visuo-spatial learning and memory. For more than 25 years the MWM has been the task most extensively used and accepted by behavioral physiologists and pharmacologists. A cursory literature search revealed that well over 2500 journal articles have been published since 1982 in which this model (or variations of the model) was used to assess and compare spatial learning and memory in rodents. The MWM, while simple at first glance, is a challenging task for rodents that employs a variety of sophisticated mnemonic processes. These processes encompass the acquisition and spatial localization of relevant visual cues that are subsequently processed, consolidated, retained, and then retrieved in order to successfully navigate and thereby locate a hidden platform to escape the water (Terry Jr., 2009).

Various objects were placed in the testing room or hung on the wall so that the mice could use these visual cues as a means of navigating in the maze. With each subsequent entry into the maze the mice progressively become more efficient at locating the platform, thus escaping the water by learning the location of the platform relative to the distal visual cues. The learning curves are thus compared between groups.

Statistical Analysis:

Values for the results were expressed as mean ± SEM. The statistical analyses were done using the analysis of variance (ANOVA) and the post/hoc Neumann Keul's test. The computer softwares used were Microsoft excel 2007 edition and SPSS 10.0 for windows. Differences between means were considered significant at $P \leq 0.05$.

RESULTS

Figure 1 shows the Index of habituation (during the NORT) in the mice administered with low and high doses of ethanolic extract of *C. lutea* root and the control. Habituation is a decrease in response to stimulus after repeated presentations, which is a form of learning. The result shows a significantly lower

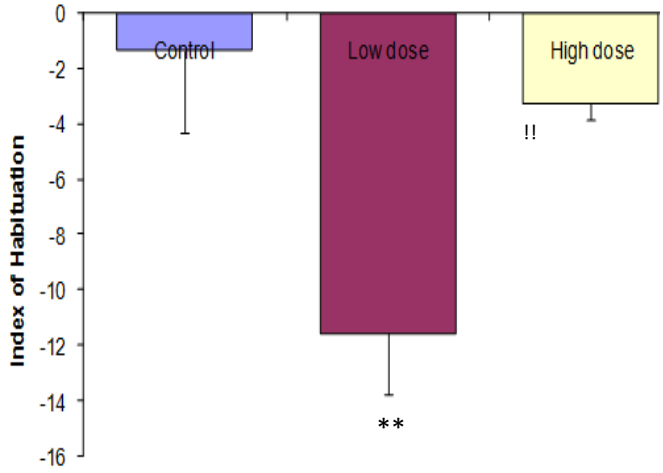

Fig 1. The Index of Habituation in the Novel Object recognition task (small Open field) in mice treated with low and high doses of ethanolic extract of *Carpolobia lutea* root and the control. ** - p< 0.01 versus control; !! – p< 0.001 versus low dose.

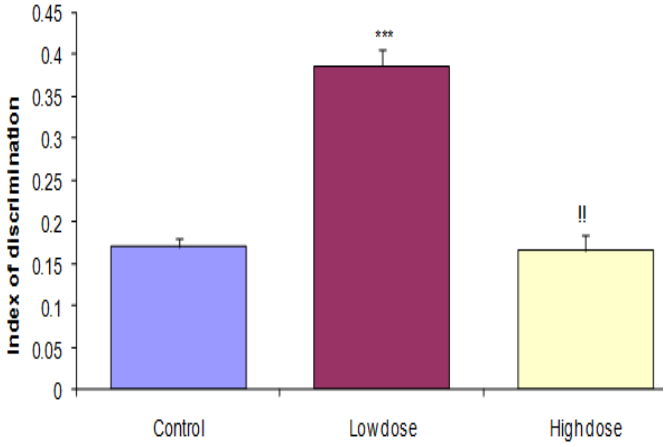

Fig 2. The index of discrimination in mice treated with low and high doses of ethanolic extract of *Carpolobia lutea* root and the control. ** - p< 0.01 versus control; !! – p< 0.001 versus low dose.

index of habituation in the low dose group (-11.570±2.219) compared to the control (-1.332 ± 2.953; p< 0.01). The index of habituation in the high dose group of mice (3.272 ± 0.564) did not differ from control but it was significantly higher than the low dose (p<0.01).

The result in Fig. 2 shows that the index of discrimination (during the NORT) in mice fed orally with the low dose of *C. lutea* extract (0.387 ± 0.019) was significantly higher than control (0.170 ± 0.010). However, the index of discrimination in the high dose group of mice (0.166 ± 0.019) did not differ significantly from control, but it was lower than that for the low dose (p<0.01).

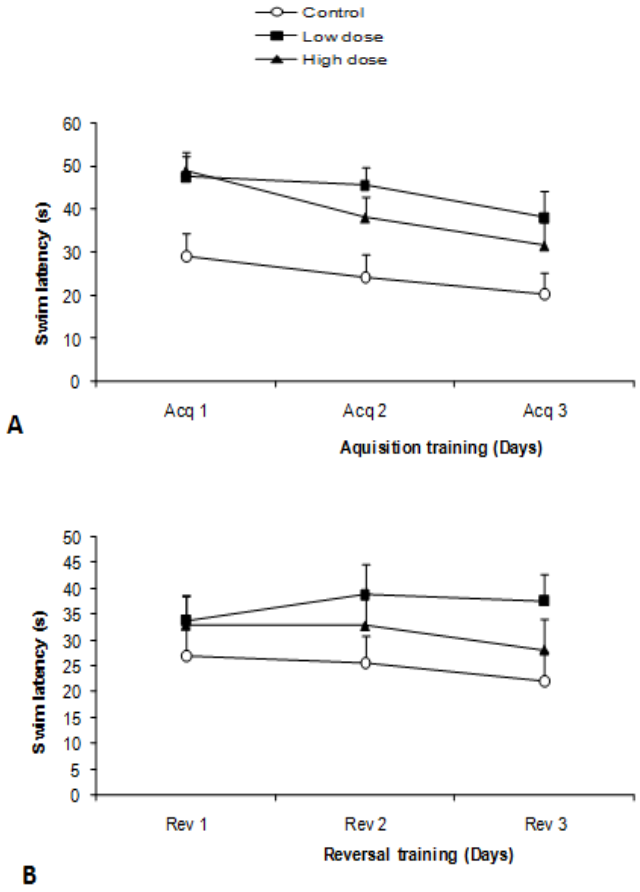

Fig 3. Swim latencies during the acquisition (A) and reversal (B) training in the Morris water maze, in mice treated with high and low dose of ethanolic extract of *Carpolobia lutea* root and the control.

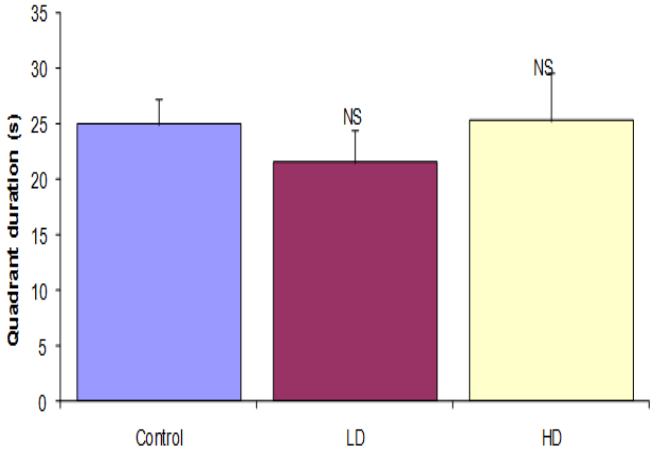

Fig 4. Quadrant duration in mice treated with low and high doses of ethanolic extract of *Carpolobia lutea* root and the control in the North-East quadrant during probe trial of the Morris water maze. NS – not significant.

The learning curve showing the swim latencies, during Acquisition (days 1-3) and Reversal (days 4-6) training in the MWM, for mice administered with low and high doses of ethanolic extract of *C. lutea* root compared to control is shown in Fig. 3. The test

groups of mice seemed to show longer swim latencies compared to controls during the acquisition training, the analysis of variance did not show any significant differences in swim latencies both in the acquisition and reversal training. Fig 4 compares the quadrant duration between the test groups and the control in the MWM. The quadrant duration for the control, low dose and high dose groups were (24.968 ± 2.363), (21.513 ± 2.882) and (25.263 ± 4.340) respectively. The quadrant duration did not also differ significantly between the groups.

DISCUSSION

The result in the novel object recognition task showed a lower index of habituation in the group of mice given a low dose of *C. lutea* root extract compared to the control. In this experimental protocol, a lower index of habituation shows that the animals spent less time investigating a familiar object, which is expected for animals which have good cognitive memory of the familiar object. Thus treatment with low dose of *C. lutea* improved cognitive memory. The index of habituation, however, did not differ following treatment with high dose of *C. lutea*, indicating that the high dose of the extract did not affect cognitive memory.

Conversely, the index of discrimination was higher in the low dose group compared to control. The index of discrimination did not differ between the high dose group and control but was lower in the high dose group compared to the low dose group. The index of discrimination shows a preference for the new (novel) object in the retention trial over the familiar object from the acquisition trial. A higher index of discrimination shows a higher preference for the novel object hence better retention and therefore better memory. The index of discrimination was higher for the group of mice treated with a low dose of *C. lutea* indicating an improved retention in this group of mice. This index however did not differ between control and the high dose indicating no change in retention and hence memory. The result in the index of discrimination is consistent with that for the index of habituation. This indicates that only the low dose of *C. lutea* improved cognitive memory in the mice. The high dose did not affect cognitive memory. It is, however, not clear why the high dose did not affect cognitive memory.

The Morris water maze tests for visuospatial learning and memory which is hippocampus dependent (Brown & Aggleton, 2001). In the MWM there was no significant difference in swim latency in the test groups compared to control during days 1-3 (Acquisition training) and days 4-6 (Retention training). Although the test groups of mice seemed to show longer swim latencies compared to controls during the acquisition training, the analysis of variance did not show any significant differences in swim latencies both in the acquisition and reversal training. This indicates that mice in all the groups were able to learn the position of the platform. The longer swim latencies in the test groups of mice may be due to the ability of the extract to decrease locomotor activity (Beshel & Nneli, 2012).

There also was no significant difference in quadrant duration in the test groups compared to control during the probe trial (Day 7) indicating that visuospatial memory was not affected by the extract of C. lutea.

The mechanism of action of the ethanolic extract of *C. lutea* root on learning and memory is not certain. It probably exerts its action via its phytochemical constituents. For instance, Saponins have been shown to improve memory of aging mice induced by D-galactose (Zhong, 2007). They may also enhance the learning and memory capacities in rats with dementia, presumably in relation to their actions to promote the scavenging of free radicals i.e. anti-oxidant property (Ouyang et al, 2005).

Flavonoids also have been implicated in reversing the course of neuronal and behavioural aging. For example, the flavanol (-) epicatechin, especially in combination with exercise, has been observed to enhance the retention, but not the acquisition, of rat spatial memory in water maze tasks (Van Praag et al, 2007). Interestingly, in that study, increased angiogenesis and neuronal spine density specifically in the Dentate Gyrus of the hippocampus was also observed. Emerging evidence suggests that flavonoid-rich foods and pure flavonoids are able to affect several different aspects of learning and memory, for example, rapid and slow acquisition, short-term working memory, long-term reference memory, reversal learning and retrieval (Rendeiro et al, 2009).

In conclusion, the ethanolic extract of *C. lutea* root enhanced cognitive memory at a low dose. It is not clear why the extract does not enhance cognitive memory at a high dose. The exact mechanism of action of the extract is not also clear. The effect of the extract on learning and memory may be mediated by Saponins (via their anti-oxidant property) and flavonoids present in the extract. This leaves a gap to be filled in future research.

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