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## Letters to the Editor / Cartas al Editor

# THE USE OF ELEVATED PLUS MAZE TO STUDY THE EFFECTS OF AQUEOUS EXTRACT OF *GARCINIA KOLA* (Linn) ON THE ANXIETY STATUS OF MALNOURISHED MICE

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### To the Editor:

*Garcinia kola* seeds are chewed as a masticatory substance to stimulate the flow of saliva, and widely consumed as snack<sup>1</sup>. Unlike other kola nuts (*Kola nitida*, *Kola acuminata*), *Garcinia kola* is thought to clean the digestive system, without abdominal problems, even when a lot of the nuts are eaten<sup>1</sup>. *Garcinia kola* is culturally very important for the Yoruba and Igbo tribes of Nigeria and for many other people living in the sub-saharan Africa. For centuries the nuts have been an important part of their lives from birth to death. They are used in traditional ceremonies, marking special events like births, marriages and conferring chieftaincy titles. A *Garcinia kola* nut tree may be planted when a baby is born with the child becoming its life long owner. In proposals of marriage, young men offer *Garcinia kola* nuts to the father of the bride, and an exchange of *Garcinia kola* nuts is essential in many business dealings as well<sup>2</sup>.

The quest for naturally occurring compounds of herbal or plant origin that could be of benefit as contraceptive and fertility control agents stimulated the interest of Isawumi<sup>3</sup> in *Garcinia kola*, seeds which are widely consumed as a stimulant. The traditional African medicinal uses include treatment of cough, purgative, anti-parasitic and anti-microbial<sup>4,5</sup>. The seed is used in the treatment of diarrhoea<sup>6</sup> bronchitis and throat infections<sup>7,8</sup> and liver disorder<sup>9</sup>. The *Garcinia kola* seeds enjoy a folk reputation in Africa as a poison antidote<sup>10</sup>. In addition, the plant possesses anti-hepatotoxic<sup>11,12</sup>, antioxidant<sup>13</sup>, hypoglycemia<sup>9,14</sup> and aphrodisiac properties<sup>15</sup>. *Garcinia kola* seeds have been reported to have an anti-inflammatory activity<sup>4,16</sup>. These studies have revealed that the process of ovulation is comparable to an inflammatory process<sup>17</sup>. Anti-inflammatory drugs have been employed in blocking ovulation<sup>18</sup>.

Some flavonoids (including apigenin based) suppress the formation of cyclo-oxygenase-2 enzyme thus playing an important role in the prevention of cancer and inflammation, partly via inhibiting cox-2 enzymes. This property is also currently under trial in chemoprevention potentials against human cancers as many types of cancer cells use cox-2 to propagate<sup>17</sup>.

*Garcinia kola* seeds contain biflavonoid capable of having anti-inflammatory properties<sup>11</sup> and are a natural antioxidant<sup>13,19</sup>. Constituents of the seed of *Garcinia kola* include 1 - 3, 8 - 11 benzophenones, *Garcinia* biflavonones (GB-1, GB- 2) and kola flavonone<sup>20</sup>. Apigenin based flavonoids represent 60% of the total flavonoids present in the diethyl ether fraction of *Garcinia kola* seed<sup>21</sup>. Phenolic compounds likely to be present in *Garcinia kola* are mostly secondary plant metabolites like tannin, saponin, oxalates etc. High consumption of these compounds is therefore dangerous to health. In conclusion, pharmacologic importance of *Garcinia kola* cannot be ruled out all the same high quantity of *Garcinia kola* should not be consumed at a time considering the adverse effects of high saponin, cyanogenic glycoside and other glycoside<sup>20</sup>.

Malnutrition is a general term for the medical condition caused by an improper or insufficient diet. It most often refers to undernutrition resulting from inadequate consumption, poor absorption, or excessive loss of nutrients, but the term can also encompass overnutrition, resulting from over eating or excessive intake of specific nutrients. An individual will experience malnutrition if the appropriate amount of, or quality of nutrients comprising of healthy diet are not consumed for an extended period of time. An extended period of malnutrition can result in starvation<sup>22</sup>. Malnutrition, as the lack of sufficient nutrients to maintain healthy bodily functions, is typically associated with extreme poverty in economically developing countries. It is a common cause of reduced intelligence in parts of the world affected by famine such as Ethiopia<sup>23</sup>. Malnutrition as the result of inappropriate dieting, overeating or the absence of a "balance diet" is often observed in economically developed countries (e.g. as indicated by increasing levels of obesity). Most commonly, malnourished people either do not have enough calories in their diet, or are eating a diet that lacks protein, vitamins, or trace minerals. Medical problems arising from malnutrition are commonly referred to as deficiency diseases. Scurvy is a well-known and now rare form of malnutrition, in which the victim lacks vitamin C.

Thirty-two adult mice with average weight of 20g were used. They were bred in the Animal Holdings of the Department of Anatomy and Cell Biology, Obafemi Awolowo University, Ile-Ife, Nigeria. Four plastic cages were procured for the four different groups of the animals. Proper care of the animals was based on Leader<sup>24</sup> and the National Institute of Health (U.S. PHS policy) on humane care of laboratory animal<sup>25</sup>.

The malnourished feed with which the animals were fed was prepared in the Histology Laboratory of the Department of Anatomy and Cell Biology, Obafemi Awolowo University, Ile-Ife. The formulation of the feed is according to Robinson<sup>26</sup> and Nwoha<sup>27</sup> as stated: Grains 72g, Casein 3g, Cellulose 8g, Oil 6g, Sucrose 5g, Vitamin 6g. Accurate measurements of the parameters stated above was ensured with the use of Mettler Toledo sensitive balance. They were mixed together with water, molded and oven dried before feeding the animals with it. *Garcinia kola* nuts were bought from the local market in Ile-Ife, Nigeria. The outer coats were removed, and the seeds were cut into pieces and air-dried. The dried-seeds were ground into fine powder and cold extraction was done using distilled water in a Soxhlet extraction kit at the Science Central Laboratory of the Obafemi Awolowo University, Ile-Ife, Nigeria. 20 g of the extract was weighed out and dissolved in 100 ml of distilled water to give 200 mg/ml of aqueous extract of *Garcinia kola*.

3-Nitropropionic acid was obtained from Sigma Chemical Co. St. Louis, U.S.A. The mice in the treatment groups received 20 mg/kg body weight of 3-Nitropropionic acid intraperitoneally. The control groups received an equivalent volume of distilled water intraperitoneally. After administration the mice were left in their cages for the next three days to allow for the neurotoxic effects of the drug on the hippocampus<sup>28</sup>.

The mice were carried to the test room in their home cages and tested one at a time for 5 minutes each. Mice were handled by the end of their tails at all times. They were taken from their home cages and placed randomly into the apparatus for exploration. After the 5 minutes test, the mice were returned to their cages and the apparatus was cleaned with 70% ethanol and permitted to dry between tests.

Elevated plus maze was used to determine the anxiety level of the mice. This apparatus consists of two open arms and two arms that are enclosed by high walls. The open arms are perpendicular to the closed arms, with the four arms intersecting to form the shape of a plus sign. The elevated plus-maze is usually elevated approximately 50cm above the floor. Security is provided by the closed arms while the open arms offer exploratory value. Therefore, one might expect anxious mice to spend less time in the open arms than those that are less fearful<sup>29</sup>. The mice were placed in the centre of the elevated plus-maze and activities are recorded for 5 minutes.

With regard to the behaviour of MM, MG and MGN, one way ANOVA revealed that there was significant effect of intake of *Garcinia kola* on number of entries into close arm ( $F_{2, 16} = 5.30$ ;  $P < 0.05$ ); number of entries into open arm ( $F_{2, 16} = 5.31$ ;  $P < 0.05$ ); % entries into open arm ( $F_{2, 16} = 7.82$ ;  $P < 0.05$ ); number of head-dip in open arm, ( $F_{2, 16} = 32.08$ ;  $P < 0.05$ ); time head-dipping ( $F_{2, 16} = 29.70$ ;  $P < 0.05$ ); grooming in close arm ( $F_{2, 16} = 12.29$ ;  $P < 0.05$ ), grooming in open arm ( $F_{2, 16} = 11.93$ ;  $P < 0.05$ ); freezing in close arm, ( $F_{2, 16} = 23.01$ ;  $P < 0.05$ ); freezing in open arm ( $F_{2, 16} = 13.14$ ;  $P < 0.05$ ), but has no significant effect on number of entries at the center stage ( $F_{2, 16} = 0.93$ ;  $P < 0.05$ ) rearing in close arm ( $F_{2, 16} = 0.40$ ;  $P < 0.05$ ), rearing in open arm ( $F_{2, 16} = 0.74$ ;  $P < 0.05$ ). Going by the (SNK) test, there were significant effects on number of entries into close arm, % entries in open arm, number of head-dip in open arm, Time head-dipping, number of time at the center stage, freezing in close arm and freezing in open arm, but not significant on number of entries into open arm, grooming in open arm, grooming in close arm, rearing in open arm and rearing in close arm ( $P < 0.05$ ) when comparing MM, and MN (Table 1).

**Table 1: MEAN BEHAVIOURAL STUDIES OF THE MALNOURISHED MICE IN THE ELEVATED PLUS - MAZE**

ELEVATED PLUS-MAZE TEST	MEAN BEHAVIOURAL STUDIES			
	A n=6	B n=6	C n=6	D n=7
Nº. ENTRIES INTO CLOSE ARM	8.57 ± 1.16*§	4.50 ± 0.31§	9.50 ± 0.81*	6.71 ± 0.44*
Nº. ENTRIES INTO OPEN ARM	4.71 ± 0.98*	3.83 ± 0.37	7.50 ± 1.05*	5.14 ± 0.51*
% ENTRIES INTO OPEN ARM	35.48 ± 5.78*§	25.80 ± 1.92§	43.42 ± 3.60*	43.15 ± 2.70*
Nº. HEAD-DIP IN OPEN ARM	7.29 ± 0.80*§	15.83 ± 1.07§	24.50 ± 3.17*	37.67 ± 2.91*
TIME HEAD-DIPPING	14.43 ± 3.18*§	9.50 ± 1.12§	19.83 ± 2.23*	62.71 ± 6.85*
Nº. TIME AT THE CENTER STAGE	11.00 ± 1.28§	6.33 ± 0.51§	13.83 ± 1.44	13.86 ± 0.74
GROOMING IN CLOSE ARM	15.00 ± 1.78*	11.17 ± 4.50	8.00 ± 3.94*	9.43 ± 2.88*
GROOMING IN OPEN ARM	0.14 ± 0.13*	0.16 ± 0.15	0*	0.71 ± 0.44*
REARING IN CLOSE ARM	22.57 ± 3.17	21.83 ± 2.11	26.67 ± 4.05	24.43 ± 1.25
REARING IN OPEN ARM	0.29 ± 0.26	0	0	0.43 ± 0.19
FREEZING IN CLOSE ARM	18.86 ± 13.20*§	37.17 ± 17.81§	10.83 ± 5.82*	2.00 ± 0.88*
FREEZING IN OPEN ARM	2.29 ± 0.92*§	0§	26.17 ± 16.97*	0*

A-Malnourished Mice (MM)      B-Malnourished Mice with Neurotoxin (MN)

C-Malnourished Mice with *Garcinia kola* extract (MG)D-Malnourished Mice with Neurotoxin and *Garcinia Kola* extracts (MNG)

\* - Implies significant effect at P &lt; 0.05 when comparing MM, MG and MNG using one way ANOVA.

§ - Implies significant effect at P &lt; 0.05 when comparing MM and MN using Student Newman-Keuls (SNK) test.

The elevated plus maze has been described as a simple method for assessing anxiety responses of rodents<sup>30</sup>. A task, using a Y-shaped apparatus that included an elevated open alley, which produced a strong approach-avoidance conflict, and an enclosed alley, which did not<sup>29</sup>. This task was modified into an elevated maze with four arms (two open and two enclosed) that are arranged to form a plus shape and was described by Handley and Mithani<sup>31</sup>. These authors described the assessment of anxiety behavior of rodents by using the ratio of time spent on the open arms to the time spent on the closed arms. Unlike other behavioral assays used to assess anxiety responses that rely upon the presentation of noxious stimuli (i.e., electric shock, food/water deprivation, loud noises, exposure to predator odor, etc.) that typically produce a conditioned response, the elevated plus maze relies upon rodents' proclivity toward dark, enclosed spaces (approach) and an unconditioned fear of heights/open spaces<sup>32</sup>. There is great diversity in possible applications of the elevated plus maze. To name a few, prescreening of newly developed pharmacological agents for treatment of anxiety-related disorders can be carried out. The anxiolytic and anxiogenic effects of pharmacological agents, drugs of abuse and hormones can be investigated. The effects of reproductive senescence/aging and/or pre-, peri- or postnatal exposure to various stressors can be assessed. Furthermore, beyond its utility as a model to detect anxiolytic effects of benzodiazepine-related compounds, the elevated plus maze can be used as a behavioral assay to study the brain sites (e.g., limbic regions, hippocampus, amygdala, dorsal raphe nucleus, etc.)<sup>33,34</sup> and mechanisms (e.g., GABA, glutamate, serotonin, hypothalamic-pituitary-adrenal axis neuromodulators, etc.)<sup>35</sup> underlying anxiety behavior. Indeed, the elevated plus maze has been used as a model of state, unconditioned anxiety for over two decades.

Behavioral responses in the elevated plus maze are easily assessed and quantified by an observer. Briefly, rodents are placed in the intersection of the four arms of the elevated plus maze and their behavior is typically recorded for 5 min. This was based upon the early studies by Montgomery<sup>29</sup> that revealed that rats demonstrated the most robust avoidance responses in the first 5 min after placement in the elevated open alleys. The behaviors that are typically recorded when rodents are in the elevated plus maze are the time spent and entries made on the open and closed arms. Behavior in this task (i.e., activity in the open arms) reflects a conflict between the rodent's preference for protected areas (e.g., closed arms) and their innate motivation to explore novel environments. Anti-anxiety behavior (increased open arm time and/or open arm entries) can be determined simultaneously with a measure of spontaneous motor activity (total and/or closed arm entries), albeit the arm entries made in the maze may not be an optimal measure of motor activity. Other ethological measures that can be observed in rodents in the maze are the number of rears, head dips, fecal boli, freezing or stretched-attend postures.

In the elevated plus-maze, % entries into open arm was greatest in MG, followed by MNG, then by MM, and least in MN. The % open arm entries and % time spent in open arms are both measures of fear and anxiety<sup>30,36</sup>. This showed that MN were the most fearful and demonstrated greatest level of anxiety. They have least head-dips and greatest grooming which showed that they were least active. Freezing in both close and open arm were greatest in these mice which showed an evidence of being fearful, and rearing both in close and open was least in them that indicated least locomotor activity.

Considering all the behavioural activities, it could be deduced that MN were most fearful, dormant, anxious, less active, with low exploratory activity but were protected from all these by feeding with aqueous extract of *Garcinia kola*.

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