Garcinia kola improves cognitive and motor function of a rat model of acute radiation syndrome in the elevated plus maze

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We reported recently that the elevated plus maze is a good tool for evaluating cognitive and motor functional changes in gamma-irradiated rats as a model for new drug evaluation and monitoring. The capacity of Garcinia kola to mitigate radiation-induced brain injury is currently unknown. We therefore assessed the effects of the neuroprotective medicinal plant Garcinia kola, on the cognitive and motor changes in this murine model of acute radiation syndrome. Wistar rats exposed once to an ionizing dose of Tc99m-generated Gamma radiation were treated with an ethyl acetate fraction of methanolic extract of Garcinia kola seeds (content of 100 mg/kg of extract) for 9 weeks. Cognitive and motor function indicators were assessed in the elevated plus maze in these animals and compared with irradiated control groups (vitamin C- and vehicle-treated groups) and the non-irradiated control rats. The irradiated control group displayed cachexia, shaggy and dirty fur, porphyrin deposits around eyes, decreased exploratory activity, reduced social interactions and a loss of thigmotaxis revealed by a marked decrease in rearing episodes and stretch attend posture episodes close to the walls of elevated plus maze closed arm, an increased central platform time, and decreases in open arm time and entries. This group further displayed a decrease in head dips and grooming episodes. Treatment with Garcinia kola, and in a lesser extent vitamin C, significantly prevented the body weight loss ($P < 0.001$) and mitigated the development of elevated plus maze signs of cognitive and motor affections observed in the irradiated control group ($P < 0.05$). Altogether, our data suggest for the first time that Garcinia kola seeds have protective properties against the development of cognitive and motor decline in the acute radiation syndrome-like context. Future studies are warranted to characterize the molecular mechanisms and neuronal networks of this action.

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Abbreviations: EPM = elevated plus maze; GIR = Gamma-irradiated rats; LSD test = least significant difference test

Introduction

Human accidental exposure to large doses of ionizing radiations results in an intractable condition known as acute radiation syndrome, whose hallmark is characterized by a neurotoxicity syndrome marked by an inflammation-mediated encephalopathy and other debilitating and life-threatening pathologies resembling neurodegenerative disorders and ischaemic brain disease. The latter include pathologies such as those resulting from neuroinflammation and related neuronal loss, endothelial affections, and blood–brain barrier dysfunction. In large amounts, agents emitting Gamma radiations such as the commonly used imaging agent, technetium 99m (Tc99m) have been reported to cause brain damage in laboratory rodents, and acute radiation syndrome-like clinical signs were reported in such experimental exposure to Gamma radiations.

Various studies using ethological tests, and particularly the elevated plus maze (EPM) paradigm, reported changes in rodent exploratory behaviour following experimental radiation-induced brain injury. The EPM paradigm is a commonly used ethological test based on conflict between rodent aversion for open brightly-lit spaces and tendency to explore a novel environment.

We recently reported the changes in EPM indicators of CNS disease in rodents exposed to gamma radiation. We observed decreases in arm transitions, in the distance covered in the maze, in open arm time and entries, in head dipping and grooming episode number, in rearing episodes and stretch attend posture episodes, in the amount of urine released, and increases in faeces emitted by irradiated rats. These findings corroborate previous reports of increases in behavioural indicators of motor and cognitive affections in irradiated rodents. However, the capacity of Garcinia kola to mitigate radiation-induced brain injury is currently unknown.

Considering that therapeutic agents are urgently needed in the field for acute radiation syndrome but also for the neurological component of late complications of radiotherapy treatment, we assessed the effects of seeds of the medicinal plant G. kola (Guttiferae) on the development of cognitive and motor alterations typically observed in gamma-irradiated rats (GIR), a murine model of acute radiation syndrome. Generally referred to locally as ‘bitter kola’ because of its bitter taste or ‘male kola’ because of its other yet to be proven virtues, G. kola seeds have been used in African traditional medicine for treating various ailments, including liver and metabolic disorders, hepatitis, diarrhoea, laryngitis, bronchitis and...
The methanolic extract of *G. kola* seeds, in particular the ethyl acetate fraction, was reported to have neuroprotective and antioxidant properties.25–29

### Material and methods

#### Animals and procedures

Forty-four male Wistar albino rats (194–200 g) were obtained from the animal house of the Faculty of Medicine and Biomedical Sciences (FMBS) of the University of Yaoundé I (Yaoundé, Cameroon) and housed at the Neuroscience Laboratory, FMBS, under natural day–night cycle, at 25°C. They had ad libitum access to normal rat chow and tap water.

Animals were randomly divided into four groups (*N* = 11 per group): a non-irradiated group given ethanol 50% (vehicle solution) (non-irradiated control group) and three groups irradiated once with gamma radiation.19 For 9 weeks after irradiation, the animals of the irradiated groups were given (daily, *per os*) *G. kola* extract in ethanol 50% (*G. kola*-treated group), ethanol 50% (irradiated control group) or vitamin C in distilled water (2.5 mg/kg)30 (vitamin C-treated or positive control group). Animals of the non-irradiated group were given the vehicle solution (non-irradiated control group). For each animal, the volume of solution to administer was calculated as follows:

\[
\text{Volume (l)} = \frac{\text{Dose (mg/kg)} \times \text{Animal weight (kg)}}{\text{Ponderal concentration (mg/ml)}}
\]

Cognitive and motor function impairment induced by irradiation and the effects of 9 weeks’ treatments were evaluated using the EPM behavioural test as earlier reported.19 Body weight was measured twice a week for each animal.

All the experimental procedures were approved by the institutional ethics committee. Animals were handled following ethical rules on the protection of animals used for scientific purposes, particularly European Commission directive (2010/63/EU).

#### Plant processing and extract preparation

*Garcinia kola* seeds were harvested during maturing period (August) in Bamenda, North West region of Cameroon. Seeds were authenticated by the National Herbarium of Cameroon and the department of Botany of the University of Yaoundé I, and a sample was stored (specimen N° 28837/HNC). Seed coats were peeled off and seeds were cut into small pieces and shade-dried at laboratory temperature. Dried seeds were ground into powder using a grinding mill. The powder (2500 g) was mixed and extracted with methanol at 65°C (5 h) using a Soxhlet extractor. Then, 100 ml of distilled water and 150 ml of ethyl acetate were added to the methanolic extract in a decantation ball. After 10 min, the dark organic phase was separated from the aqueous phase and collected. An additional 150 ml portion of ethyl acetate was added to the decantation ball and after 10 min, the organic phase was separated once again from the aqueous phase and collected. The process was repeated until the organic phase became less dark than the aqueous phase. The organic phases collected were mixed and dried using a rotatory evaporator at 70°C. The dried extract of *G. kola* (ethyl acetate fraction of methanolic extract, termed as *G. kola* extract in this manuscript) weighed 153.86 g (yield of the extraction: 6.2%). It was diluted in ethanol 50% and administered *per os* at 40 mg/ml to correspond to the content of a dose of 100 mg/kg of methanolic extract of *G. kola* seeds, considering that this dose of extract was reported to have strong antioxidant and neuroprotective effects in both our previous works31,32 and reports of other groups.27,28,33

#### Gamma radiation exposure

The rats were irradiated by overexposure to large amount of technetium 99m (Tc99m) Gamma radiation as previously described.19 The targeted brain radiation absorption was 667 mGy, as absorptions ranging from 500 to 1000 mGy were reported to cause brain lesions.9,10 Briefly, 10 ml of a solution of pertechnetate was eluted from Tc99m-generator and a syringe of 1110 MBq of Gamma activity was prepared for each rat, corresponding to an absorbed dose of 667 mGy (66.7 Rad). The volume of radioactive Tc99m administered through the tail vein to each rat was 0.16 ml. Rat irradiation was done in the Radiotherapy unit of the Yaoundé General Hospital.

#### EPM test

The EPM apparatus was elevated at 50 cm above the floor and consisted of two open arms (50 × 10 cm) crossed at a central platform (right angles) with two opposed arms of the same size enclosed by walls (40 cm high), with squares drawn on the floor. Each rat was placed on the central platform of the apparatus facing an open arm and was allowed to explore the maze for 5 min. Animal performance in the EPM was recorded using a computerized video recording system with a camera placed 150 cm above the centre of the apparatus. After 5 min, rats were returned to their home cage. After each trial, the walls and the floor of the arms and the floor of the central platform of the maze were cleaned with a 70% ethanol solution to prevent bias due to olfactory cues.

Video recordings were analysed offline using the Limelight Video Tracking System (Bilaney Consultants, Duesseldorf, Germany). Arm entries and the time in each arm were determined. An arm entry was counted when all the rat paws were in the arm. In addition, episodes of
rearing (when an animal stood upright on hind limbs), head dipping (when an animal lowered its head over a side of the open arm towards the floor), grooming (when an animal licked and scratched itself for more than 3 s while stationary) and stretch attend posture (animal forward elongation of head and shoulders followed by retraction to original position) were counted. The total distance covered in the maze and the surface area of the urine released on the maze (puddles or streaks) floor were determined.

**Statistical analysis**

Changes in body weight in the irradiated group given vehicle solution (irradiated control group) were compared to changes in the irradiated groups treated with *G. kola* extract or vitamin C, and to changes in the non-irradiated control group in each week post-treatment, using one-way ANOVA followed by Fisher’s least significant difference (LSD) post hoc test. Differences in EPM performances at the end of the treatment were also compared between *G. kola*- and vitamin C-treated irradiated groups, the non-irradiated control group, and the irradiated control group using one-way ANOVA followed by LSD test. Differences with *P* < 0.05 were considered significant. Data were presented as mean ± standard deviation.

Our data are available as Supplementary material.

**Data availability statement**

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

**Results**

**Animal condition**

Treatment with *G. kola* improved the general animal condition and prevented the systemic disease signs observed in the irradiated control group, such as cachexia, porphyrin deposits around eyes, shaggy and dirty fur, decreased exploratory activity, and reduced social interactions. During handling every 3 days to change cage litter and to perform EPM tests, none of the animals expressed the common laboratory rodent marker of pain vocalization. Figure 1 shows body weight changes of irradiated animals receiving the vehicle solution, treatment with *G. kola*, treatment with vitamin C, and of non-irradiated animals. Irradiation slowed the increase in body weight (*y* = 2.41x + 11.17, *R*² = 0.96) compared to the non-irradiated group (*y* = 5.17x + 17.83, *R*² = 0.88), with marked differences from post-irradiation week 2 (*P* = 0.007). Improvements were observed in irradiated animals treated with *G. kola* (*y* = 4.86x + 17.11, *R*² = 0.89), and in a lesser extent, vitamin C (*y* = 3.33x + 10.61, *R*² = 0.98).

The data that support the findings of this study are available as Supplementary material. Our data are available as Supplementary material.

**Arm entries and time in the EPM**

Figure 2 shows arm entries and time in the EPM of irradiated animals receiving the vehicle solution, treated with *G. kola*, treated with vitamin C, and of non-irradiated animals. Compared to the non-irradiated group, irradiated animals given the vehicle displayed significant decreases in open arm time (*P* = 0.014) (Fig. 2A), central platform time (*P* = 0.0006) (Fig. 2C), open arm entries (*P* = 0.022) (Fig. 2D), closed arm entries (*P* = 0.0005) (Fig. 2E) and total arm transitions (*P* = 0.0002) (Fig. 2F). Conversely, closed arm time was decreased (*P* = 0.002) (Fig. 2B). Vitamin C treatment prevented changes in open arm time (*P* = 0.043) and arm transitions (*P* = 0.0053) (Fig. 2A and F). On the other hand, *G. kola* treatment prevented or mitigated changes in open arm time (*P* = 0.031) (Fig. 2A), central platform time (*P* = 0.019) (Fig. 2C), arm entries (0.033) (Fig. 2D) and arm transitions (0.0053) (Fig. 2F). No statistically significant difference was observed for these parameters between *G. kola*-treated and non-irradiated animals (Fig. 2A–F).

**Exploratory behaviour indicators in the EPM**

Figure 3 shows the main exploratory behaviour indicators revealed by the EPM of irradiated animals receiving the vehicle solution, treated with *G. kola*, treated with vitamin C.
vitamin C, and of non-irradiated animals. Compared to non-irradiated, irradiated animals given the vehicle displayed significant decreases in the distance covered in the maze ($P = 0.000001$) (Fig. 3A), the rearing episode number ($P = 0.000001$) (Fig. 3B), the grooming episode number ($P = 0.00002$) (Fig. 3C), the stretch attend posture episode number ($P = 0.000001$) (Fig. 3D), the head dipping episode number ($P = 0.00002$) (Fig. 3E) and the urine-wet area ($P = 0.0104$) (Fig. 3F). Vitamin C and *G. kola* treatments prevented decreases in the distance covered ($P = 0.033$ and $P = 0.0002$, respectively, compared to non-irradiated group) (Fig. 3A), the rearing episode number ($P = 0.028$ and $P = 0.0007$, respectively) (Fig. 3B), the grooming episode number ($P = 0.0008$ and $P = 0.00003$, respectively) (Fig. 3C), and the stretch attend posture episode number ($P = 0.0005$ and $P = 0.0003$, respectively) (Fig. 3D). *Garcinia kola* treatment also prevented decreases in head dipping episode number ($P = 0.03$) (Fig. 3E).

**Discussion**

The findings of this study suggest that *G. kola*, a medicinal plant with established neuroprotective and antioxidant properties, prevented the body weight loss and mitigated the typical cognitive and motor impairment in rats exposed once to a brain-damaging dose of Tc99m-generated Gamma radiation reported to cause. The results further highlighted the EPM as a good tool for evaluating cognitive and motor changes in the acute radiation syndrome-like context.

Notably, treatment with *G. kola* improved the animal general condition and prevented the systemic clinical signs previously reported in this model and also observed in the irradiated control group in the present study, such as cachexia, shaggy and dirty fur, porphyrin deposits around eyes, decreased exploratory activity, and reduced social interactions. Although, both *G. kola* and the antioxidant agent vitamin C increased the animal body weight, *G. kola* effect was stronger with non-irradiated group-like values, suggesting that *G. kola* effects were not only due to its well-established antioxidant properties.

Furthermore, in this study, animals of the irradiated control group displayed marked alterations in the EPM indicators of motor and cognitive functions. Notably, a loss of thigmotaxis, a robust cognitive function indicator in rodents, was revealed in these animals by a marked decrease in rearing episodes and stretch attend
posture episodes close to the walls of EPM closed arm. An increase in central platform time, which typically indicates an impairment in animal’s ability to choose the arm to explore,14,15 was observed in these animals. Moreover, decreases in arm transitions and in the distance covered in the maze, which are typical EPM indicators of cognitive and motor impairment,14–18 were also observed in animals of the irradiated control group in this study. On the other hand, G. kola treatment, and in a lesser extent, vitamin C treatment, prevented the increase in central platform time and mitigated the decreases in arm transitions, in the distance covered in the maze, and in rearing episodes and stretch attend posture episodes close to the walls of EPM closed arm. These findings suggest that G. kola treatment prevented or mitigated the development of cognitive and motor impairment in GIR.

Besides, decreases were observed in open arm time and entries, in head dipping episode number, and in the amount of urine released during the test, i.e. in EPM standard indicators of anxiety-like mood,14–18 in irradiated control group. Irradiated control group animals also had shaggy and dirty fur, and displayed a marked decrease in the number of grooming episodes, all indicators of depression-like mood.5,7 Interestingly, treatment of irradiated animals with either vitamin C or G. kola significantly mitigated the development of these pathologic signs. With an overall stronger response than vitamin C, treatment of irradiated animals with G. kola prevented decreases in open arm time and in the number of grooming episodes, mitigated the decreases in arm entries and head dipping episode number, and increased slightly the amount of urine released during the test. Altogether, our observations in GIR corroborate previous reports from behavioural studies in irradiated rodents where decreases in motor activity and increases in indicators of anxiety-like mood were observed.11–13 Finally, our data suggest that G. kola treatment improved the general condition of the animals as well as motor and cognitive functions in GIR.

Further studies are needed to elucidate the mechanisms by which the observed action by G. kola is exerted. Such studies could evaluate the effects at varying doses of G. kola extracts. Future studies will also extend the current assessment to evaluate the potential of G. kola in...
mitigating toxicities due to external beam radiotherapy of brain tumours or metastasis, which could be highly valuable in radiation oncology.

**Conclusion**

We assessed the effect of the ethyl acetate extract of seeds of *G. kola* on EPM cognitive and motor indicators in rats exposed once to a brain-damaging dose of Tc99m-generated Gamma radiation, a murine model of acute radiation syndrome. The *G. kola* seed extract prevented the body weight loss and mitigated the typical EPM indicators of cognitive and motor impairment in GIR better than vitamin C, suggesting that these effects were only partly due to the established antioxidant properties of seeds of this medicinal plant. Further fractionation and mechanism studies are warranted to unravel the active principles and pathways accounting for the mitigation of decreases in cognitive and motor functions by *G. kola* seed extract in acute radiation syndrome-like context.

**Supplementary material**

Supplementary material is available at *Brain Communications* online.

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**Competing interests**

The authors report neither competing interests nor any conflict of interest concerning the work in this paper.

**References**

1. Happold C, Ernemann U, Roth P, et al. Anticoagulation for radiation-induced neurotoxicity revisited. J Neurooncol. 2008;90(3):357–362.
2. Behin A, Delattre JY. Complications of radiation therapy on the brain and spinal cord. Semin Neurrol. 2004;24(4):403–417.
3. Erkkonen MG, Kim MO, Geschwind MD. Clinical neurology and epidemiology of the major neurodegenerative diseases. Cold Spring Harb Perspect Biol. 2018;10(4):a033118.
4. Kalana RN, Akinyemi R, Ibara M. Stroke injury, cognitive impairment and vascular dementia. Biochim Biophys Acta. 2016;1862(5):915–925.
5. Song X, Liu B, Cui L, et al. Silibinin ameliorates anxiety/depression-like behaviors in amyloid beta-treated rats by upregulating BDNF/TrkB pathway and attenuating autophagy in hippocampus. Physiol Behav. 2017;179:487–493.
6. Pinz MP, Dos Reis AS, Vogt AG, et al. Current advances of pharmacological properties of 7-chloro-4-(phenylselenyl) quinone: Prevention of cognitive deficit and anxiety in Alzheimer’s disease model. Biomed Pharmacother. 2018;105:1006–1014.
7. Khan A, Shal B, Naveed M, et al. Matrine ameliorates anxiety and depression-like behaviour by targeting hyperammonemia-induced neuroinflammation and oxidative stress in CCl4 model of liver injury. Neurotoxicology. 2019;72:38–30.
8. Guignet M, Dhakal K, Flannery BM, et al. Persistent behavior deficits, neuroinflammation, and oxidative stress in a rat model of acute organophosphate intoxication. Neurobiol Dis. 2020;133:104431.
9. Duran A, Duran E, Castro J. [Diagnosis of brain death scintigraphy with HMPAO-TC99m]. Neurologia. 2003;18(7):389.
10. Zolotov VA, Lomanov MF, Luk’ashin VE, et al. Experimental study of the mechanism of local radiation damage to the brain by means of penetration of 99mTc through the hematoencephalic barrier. Med Radiol (Mosk). 1982(7):53–58.
11. Norton S, Mullenix P, Culver B. Comparison of the structure of hyperactive behavior in rats after brain damage from x-irradiation, carbon monoxide and palilidal lesions. Brain Res. 1976;116(1):49–67.
12. Peng Y, Lu K, Li Z, et al. Blockade of Kv1.3 channels ameliorates radiation-induced brain injury. Neuro Oncol. 2014;16(4):528–539.
13. Thabet NM, Moustafa EM. Protective effect of rutin against brain injury induced by acrylamide or gamma radiation: Role of PI3K/AKT/GSK-3beta/NRF-2 signalling pathway. Arch Physiol Biochem. 2018;124(2):185–193.
14. Vurali D, Wattiez A-S, Russo AF, et al. Behavioral and cognitive animal models in headache research. J Headache Pain. 2019;20(1):11.
15. Castanheira L, Ferreira MF, Sebastião AM, et al. Anxiety assessment in pre-clinical tests and in clinical trials: A critical review. Curr Top Med Chem. 2018;18(19):1656–1676.
16. Seke Etef PF, Farahna M, Khayr MAM, et al. Evaluation of the safety of conventional lighting replacement by artificial daylight. J Microsc Ultrastruct. 2017;5(4):206–215.
17. Padurariu M, Antioch I, Balmus I, et al. Describing some behavioural animal models of anxiety and their mechanisms with special reference to oxidative stress and oxytocin relevance. Int J Vet Sci Med. 2017;5(2):98–104.
18. Djogue S, Djijou Djeuba AD, Seke Etef PF, et al. Memory and exploratory behavior impairment in ovarietomized Wistar rats. Behav Brain Funct. 2018;14(1):14.
19. Njамmsi AK, Ahidjo N, Ngarka L, et al. Characterization of the cognitive and motor changes revealed by the elevated plus maze in an experimental rat model of radiation-induced brain injury. Adv Biomed Res. 2020;9:72.
20. Wilkinson GS, Tietjen GL, Wiggs LD, et al. Mortality among plutonium and other radiation workers at a plutonium weapons facility. Am J Epidemiol. 1987;125(2):231–250.
21. Beral V, Fraser P, Carpenter L, et al. Mortality of employees of the Atomic Weapons Establishment, 1951–82. Bmj. 1988;297(6651):757–770.
22. Pasquier D, Hoelscher T, Schmutz J, et al. Hyperbaric oxygen therapy in the treatment of radio-induced lesions in normal tissues: A literature review. Radiother Oncol. 2004;72(1):1–13.
23. Kelsey CR, Mukundan S, Wang Z, et al. Assessing neurotoxicity from the low-dose radiation component of radiosurgery using magnetic resonance spectroscopy. Neuro Oncol. 2010;12(2):145–152.

24. Icheku V, Onianwah F, Nwulia A. A descriptive cross-sectional study on various uses and outcomes of Garcinia kola among people of Oshimili North in the Delta State of Nigeria. Ayu. 2018;39(3):132–138.

25. Djague F, Lunga PK, Toghueo KRM, et al. Garcinia kola (Heckel) and Alchornea cordifolia (Schumach. & Thonn.) Mull. Arg. from Cameroon possess potential antismalmonellal and antioxidant properties. PLoS One. 2020;15(8):e0237076.

26. Farombi EO, Abolaji AO, Farombi TH, et al. Garcinia kola seed biflavonoid fraction (Kolaviron), increases longevity and attenuates rotenone-induced toxicity in Drosophila melanogaster. Pestic Biochem Physiol. 2018;145:39–45.

27. Adedara IA, Awogbindin IO, Anamelechi JP, et al. Garcinia kola seed ameliorates renal, hepatic, and testicular oxidative damage in streptozotocin-induced diabetic rats. Pharm Biol. 2015;53(5):695–704.

28. Farombi EO, Adedara IA, Oyenihi AB, et al. Hepatic, testicular and spermatozoa antioxidant status in rats chronically treated with Garcinia kolaseed. J Ethnopharmacol. 2013;146(2):536–542.

29. Okoko T. In vitro antioxidant and free radical scavenging activities of Garcinia kola seeds. Food Chem Toxicol. 2009;47(10):2620–2623.

30. Kim JH, Kim YC, Nahm FS, et al. The therapeutic effect of vitamin C in an animal model of complex regional pain syndrome produced by prolonged hindpaw ischemia-reperfusion in rats. Int J Med Sci. 2017;14(1):97–101.

31. Seke Etet PF, Farahna M, Satti GMH, et al. Garcinia kola seeds may prevent cognitive and motor dysfunctions in a type 1 diabetes mellitus rat model partly by mitigating neuroinflammation. J Complement Integr Med. 2017;14(3):2016–0167.

32. Farahna M, Seke Etet PF, Osman SY, et al. Garcinia kola aqueous suspension prevents cerebellar neurodegeneration in long-term diabetic rat - A type 1 diabetes mellitus model. J Ethnopharmacol. 2017;195:159–165.

33. Abarikwu SO. Kolaviron, a natural flavonoid from the seeds of Garcinia kola, reduces LPS-induced inflammation in macrophages by combined inhibition of IL-6 secretion, and inflammatory transcription factors, ERK1/2, NF-kB, p38, Akt, p-c-JUN and JNK. Biochim Biophys Acta. 2014;1840(7):2373–2381.