



Committee for Aid and Education in Neurochemistry (CAEN) CATEGORY 1B [2016]: Research Grant

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Research Topic: Neuroprotective Activities of Garcinia Kola Extract [GKE]
against 3, 4 Methylenedioxymethamphetamine [MDMA]
Induced Neurotoxicity in the Hippocampus, Cerebellum
and Frontal Cortex of Murine Models

Background

I [Joshua Owolabi] was awarded the Committee for Aid and Education in Neurochemistry (CAEN) CATEGORY 1B [2016], Research Grant for the Research supplies for use in the applicant's home laboratory at the Department of Anatomy, Ben Carson [Snr.] School of medicine, Babcock University, Nigeria. The grant was given to support the research project titled: Neuroprotective Activities of GKE against MDMA Induced Neurotoxicity in the Hippocampus, Cerebellum and Frontal Cortex of Murine Models. To this end, the project was carried out as described in the proposal followed by results analyses and presentation. The project was successful; first, in terms of its objectives, and secondly, by providing opportunities for insights and research exposure and exploration into an area of research in the field of Neuroscience that justifiably deserved attention. The funding was also adequate in carrying out the research.

Garcinia kola is an African herbal product with potential immense phytomedicinal properties, albeit not adequately explored. There is still much to be known about its neuroprotective properties from empirical research investigations. This research, thus, provided an opportunity to contribute to knowledge on the use of Garcinia kola as a natural neuroprotective agent against intoxicants.

Garcinia kola belongs to the Kingdom- Plantae; Phylum- Tracheophyta; Class- Magnoliopsida; Order- Theales; and Family- Guttiferae, with the species authority name- Heckel. Despite its enormous medicinal benefits, it has been listed on the IUCN Red List of Threatened Species. Garcinia kola has been severally reported to have antioxidant properties; it is a useful source anti-inflammatory phytochemicals. MDMA is an established neurotoxicant. It was therefore important to evaluate further the potentials of Garcinia kola extract in ameliorating MDMA induced neurotoxicity in vital parts of the brain.

Project Execution

By virtue of its objectives; the project was designed to observe the nature of MDMA effects on the hippocampus, frontal cortex and the cerebellum of rat models; to observe the neuroprotective effects of GKE on MDMA induced damage to the hippocampus, frontal cortex and the cerebellum of rat models; to evaluate the effects of dose variations on GKE neuroprotective potentials; to evaluate the effects of duration of exposure and treatment on the activities of MDMA and GKE on the structures of interest; and to investigate the neurobehavioural changes that are associated with MDMA and GKE effects on the hippocampus, frontal cortex and the cerebellum of experimental rats.



Wistar rats, *Rattus norvegicus*, were used as experimental animals. They were grouped as follows [Sixty Wistar rats, n=60; 10/Group]:

Group 1: Control I: Experimental rats of both sexes were fed *ad libitum*; to serve as the standard reference.

Group 2: GKE Low Dose Treated Rats Group; 100mg/kg daily dose of GKE for 35 days was administered to rats to observe the effects of GKE on brain parts.

Group 3: GKE High Dose Treated Rats Group; 200mg/kg daily dose of GKE for 35 days was administered to rats.

Group 4: MDMA-Treated Rats Group; 20mg/kg one time dose of MDMA was administered to rats to observe the effects of MDMA on rat's brain parts/regions.

Group 5: MDMA-Low Dose GKE-Treated; experimental rats were treated with 100mg/kg body weight GKE throughout the duration of experiment; but administered one-time 20mg/kg MDMA.

Group 6: MDMA-High Dose GKE-Treated; experimental rats were treated with 200mg/kg body weight GKE throughout the duration of experiment; but administered 20mg/kg one-time MDMA to observe its neuroprotective effects.

These objectives were achieved by observing the effects of MDMA versus GKE Effects on Cyto- and Histoarchitecture using histological techniques and the reactions of astrocytes' to MDMA versus GKE Activities on Brain Tissues using the Glial Acidic Fibrillary Protein technique. In addition to these, Biochemical Tests were used to observe the effects of MDMA versus GKE on Neurotransmitters and Marker Enzymes by assaying specific activities of Neurotransmitters: Dopamine, Glutamate and Enzymes: G-6-PDH, Cytochrome C Oxidase in the tissues. Neurobehavioural Tests were used to observe the effects of MDMA versus GKE on behaviour parameters, including: [1] Anxiety Test [2] Memory-Cognition.

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Project Outcome/Results

Scientific Findings and Contributions to Knowledge

Results showed that MDMA had neurotoxicity effects on the selected parts of the brain. Astrocyte reaction is observable in all the groups treated with MDMA relative to the control. Garcinia kola had ameliorative effects on the cerebellum against MDMA neurotoxicity; particularly by preserving the Purkinje cells. Biochemical results of the neurotransmitters and enzymes compliment these structural observations. Garcinia kola extract use did not produce any sign of deleterious effects. MDMA had deleterious effects on the frontal cortex as indicated by the relatively prominent astrocyte reactions in the cortex of the brains. There are signs that Garcinia kola use might mitigate or ameliorate the degree of effects of MDMA on the cortex; there was however not absolute protection from its use.

The results showed that garcinia kola holds promise as a phytomedicinal material with potential use for ameliorating neurotoxicity. Though certain previous reports, have shown that its antioxidant effects is largely responsible for its phytomedicinal importance; it is very important to further explore the effects of specific phytochemical groups and explore their use in various neurological conditions. Altogether, this material should be given adequate research attention to investigate thoroughly its usefulness. This research has therefore thrown light on Garcinia kola potential usefulness in ameliorating



neurological conditions including toxicities and possibly, oxidative stress related brain damages.

Skill and Experience

The Project provided me an opportunity to enhance my technical skills, especially the frozen sections-IHC protocols in general. Under the guidance of my mentor and professor; I was able to try out certain theoretical concepts in the laboratory by myself. The availability of reagents and antibodies meant for the research gave me the opportunity to carry out procedures using the materials, which were typically unavailable without the ISN Grant support.

Benefits to the Host Institution

The research provided a learning opportunity for a number of junior scientists, students and mentees who were involved. This gave them a sort of hands-on training and exposure. The varied number of activities- ranging from extracts preparation from the raw *Garcinia kola* to the administration of the extracts to the experimental animals, sacrifice and tissues preparations; for the various protocols were good opportunities for them to gain research skills and exposure. This has helped the host institution by improving these individuals' exposure to research.

Conclusion

This investigation has contributed significantly to knowledge on the potentials of *Garcinia kola*- a phytomedicinal material- on its possible use to achieve neuroprotection against toxicity in nervous tissues.

Appreciation

- **ISN:** I am most grateful to the ISN for funding this research project, and providing me the opportunity to explore the research questions towards contributing meaningfully to knowledge and solution on the subject of research.
- **Babcock University, Nigeria:** Babcock University provided me the laboratory facility to use for the research, the animal house facility where the animals were housed during research as well as the enabling environment that made the research successful.
- **Professor Ogunnaike Philip:** I am most privileged and honoured to have been guided and supervised by Professor P.O. Ogunnaike whose ability to mentor, guide and challenge a researcher's thinking is uncommon; yet who does it with such a positive attitude and genuine interest.
- **Head of Department:** Special thanks to Professor A.B.O. Desalu, the Head of Anatomy Department Babcock University where the laboratory facility that was used for this research was domiciled; he was supportive and encouraging.
- **Research Support Team:** my research support team members were amazing in helping with various activities during the research, and at various stages: Sunday Fabiyi, Sunday Olatunji, Samsom Oyewumi and John Olanrewaju were quite helpful. Also, Special thanks to Philip Adeniyi for his support.



Pictures

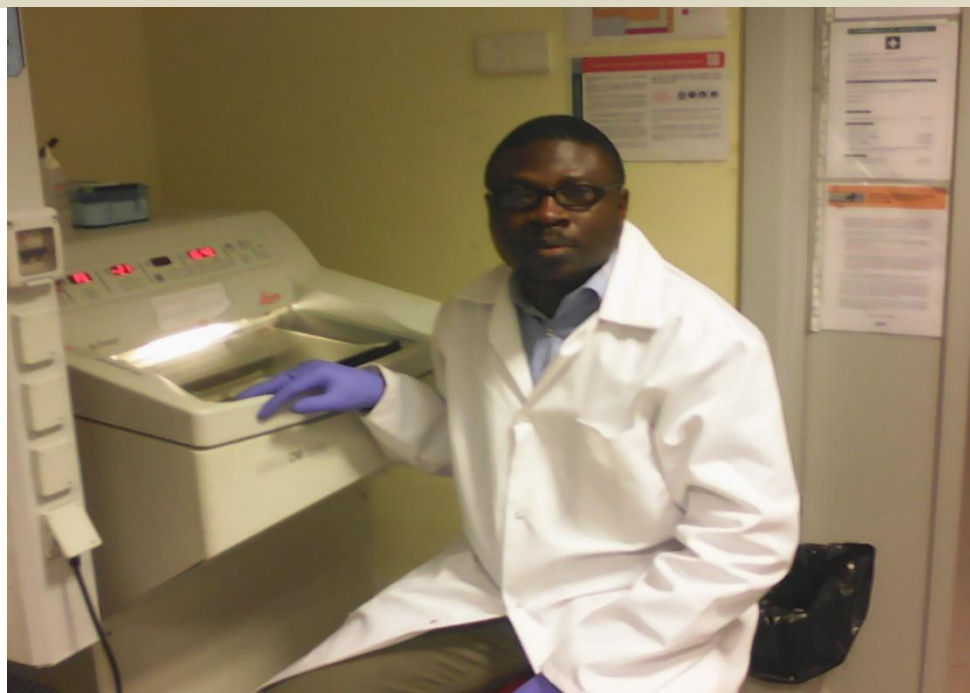


Figure 1: Dr. Owolabi Joshua, working on the cryostat in the course of the research.

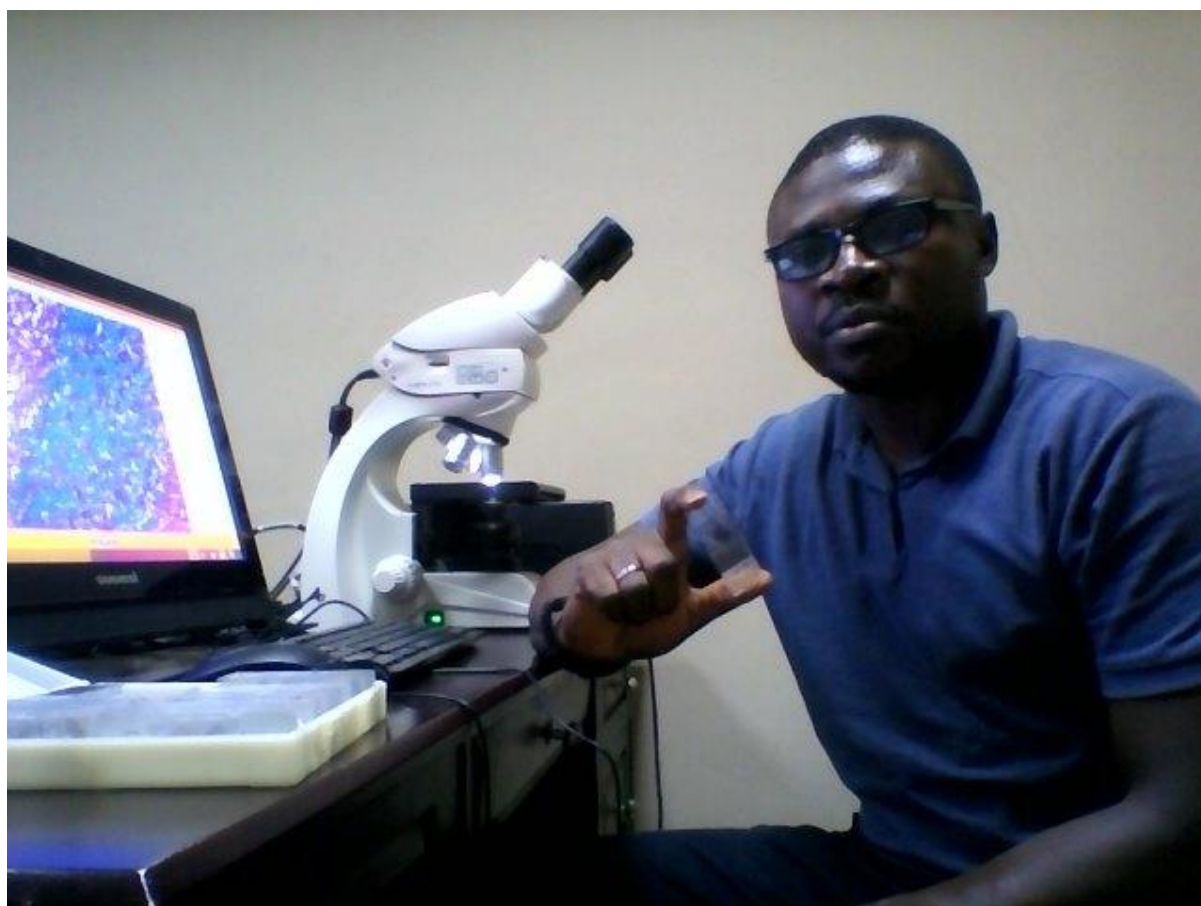


Figure 2: Dr. Owolabi Joshua working on the Digital Research Microscope in the Neuroscience Lab of Anatomy Department Babcock University, Nigeria

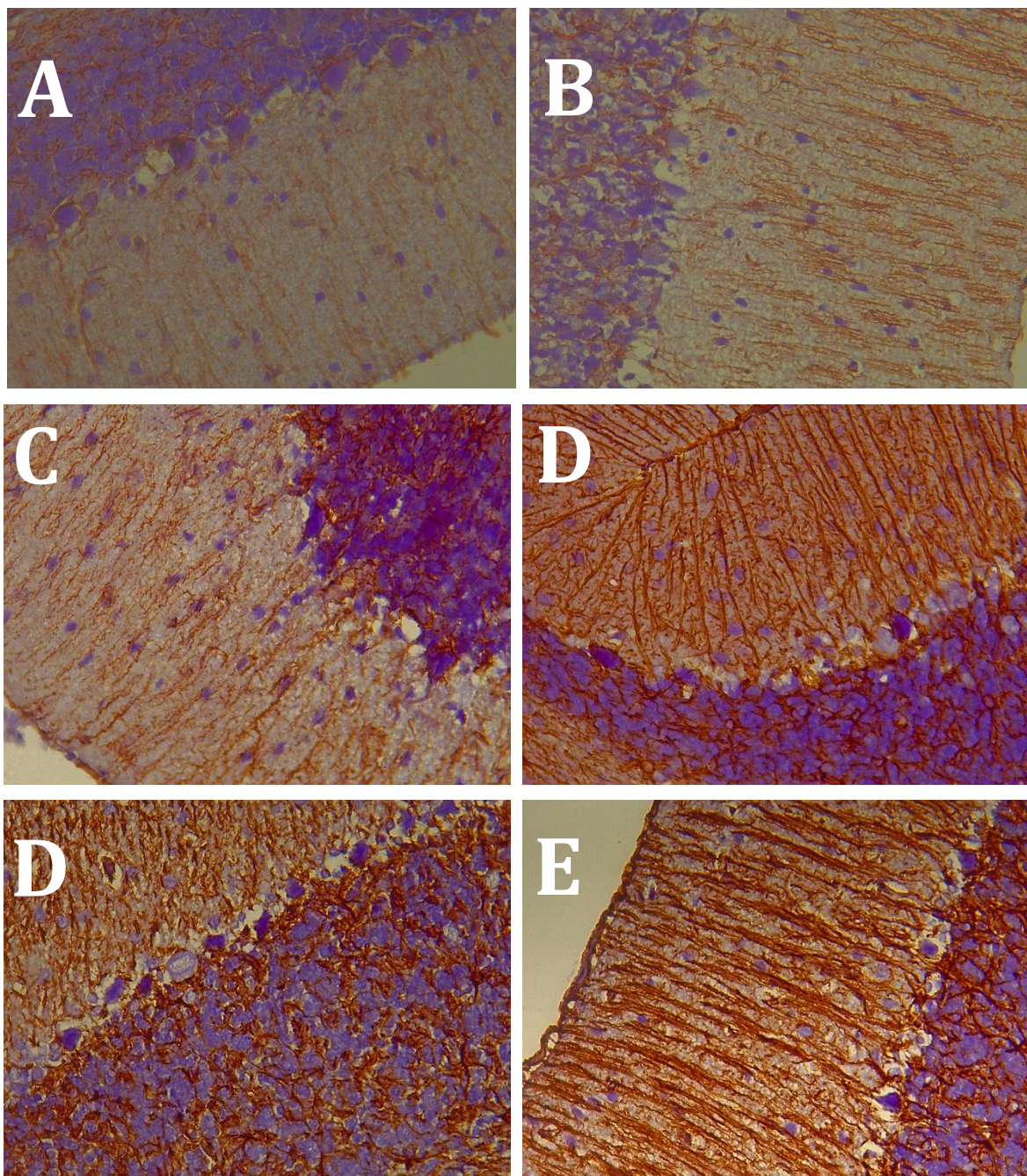
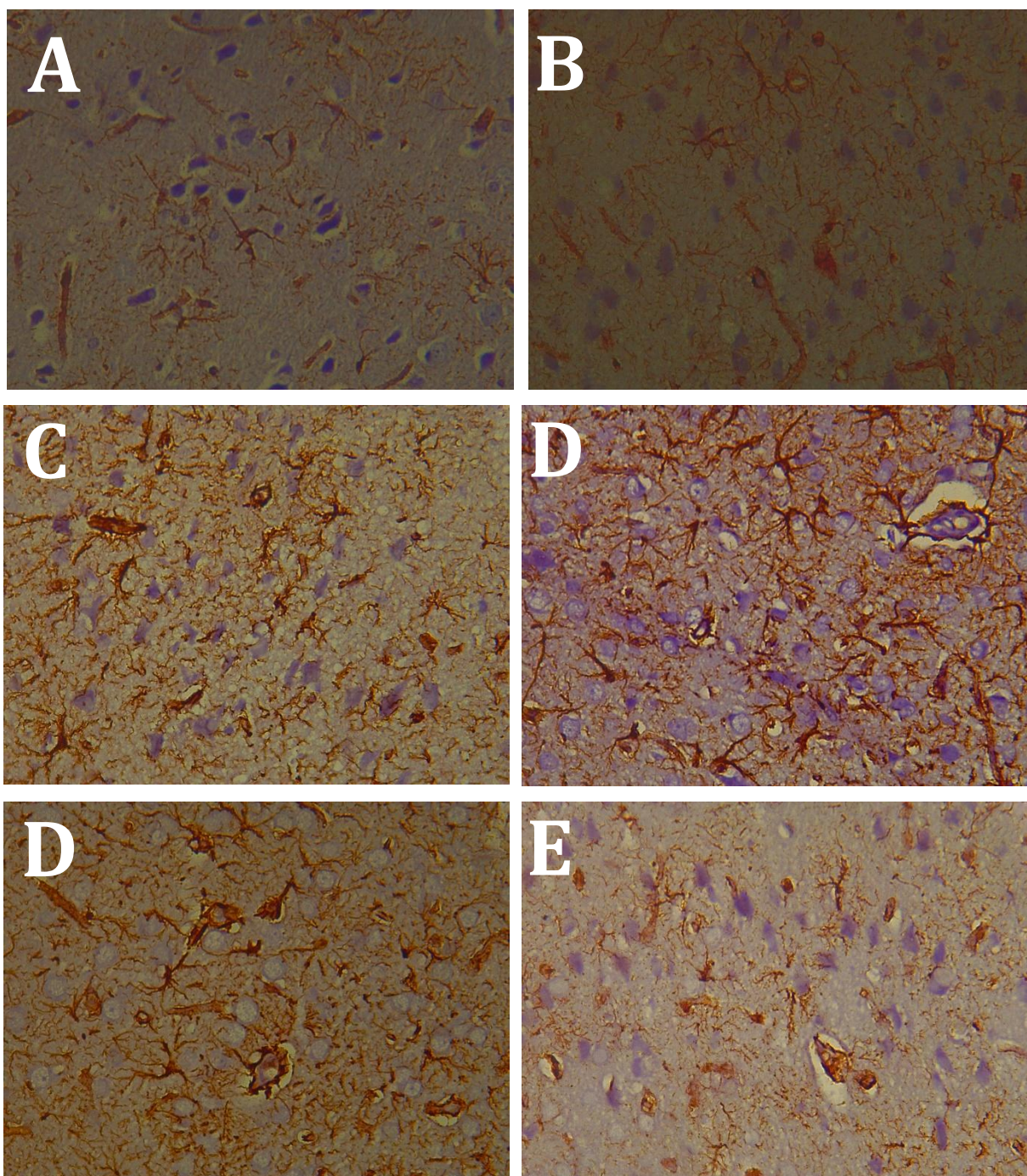
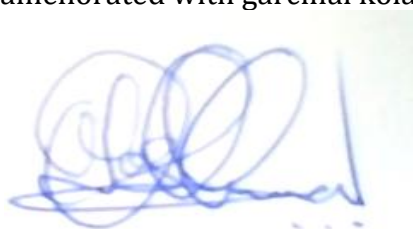


Figure 3: Photomicrograph of the cerebellum of the brain demonstrated using the Glial Fibrillary Acidic Protein [GFAP] histochemistry method to observe astrocyte reactions in the cerebellum of the experimental animals [Groups A-F]. Astrocytes reactions showed that MDMA induced neurotoxicity and garcinia kola effects could protect the tissue tissues against MDMA toxicity.



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Figure 4: Photomicrograph of the brain frontal cortex demonstrated using the Glial Fibrillary Acidic Protein [GFAP] histochemistry method to observe astrocyte reactions in the cortex of the experimental animals [Groups A-F]. MDMA induced neurotoxicity in the frontal cortex, with accompanying astrocyte reactions; the effects were relatively ameliorated with garcinal kola extract effects.



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