Research Article

The Protective Effects of Aqueous Extract of Garcinia Kola (Linn) On The Hippocampus and Cerebellum of Malnourished Mice

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Summary

The present study was undertaken to assess the neuroprotective effects of aqueous extract of Garcinia kola on neurotoxin administered malnourished mice. The study was carried out using thirty-two adult Malnourished mice which were randomly assigned into four groups (n=8): A, B, C, and D. Group A served as control, while the other groups served as the experimental groups. Animals in group A were fed malnourished diet ad libitum and water given liberally. Animals in group B were administered with 3-Nitropropionic acid (3-NP) (neurotoxin) only (20 mg/kg body weight), group C were given only Garcinia kola extracts, and group D were pre-treated with Garcinia kola extracts for seven days (200mg/kg) prior to administration of neurotoxin (20 mg/kg body weight). After three days of neurotoxins administration in the relevant groups, the brains were excised and fixed in formal calcium for histological processing.

The study showed that hippocampal and cerebellar neurons of animals in group B exhibited some cellular degeneration and blood vessel blockage, which were not seen in groups A, C and D. Cresyl violet staining was least intense in group B than in groups A, C and D. Despite the fact that animals in group D has equal administration of 3-Nitropropionic acid concentration, there were no traces of neural degeneration as it was evidenced in group B. It was concluded that Garcinia kola has protective effects on the neurons of the hippocampus and cerebellum of malnourished mice.

Key words: Garcinia kola, 3-Nitropropionic acid, Neurons, Hippocampus, Malnourished mice, Cerebellum
(200mg/Kg) ön tedavisinden sonra nörotoksin (20mg/kg vücut ağırlığı) aldılar. Geçerli gruplarda üç günlük nörotoksin verilmesinden sonra beyinler eksize edilip histolojik çalışma için formal kalsiyum içinde fikse edildi.

B grubundaki hayvanların serebellar ve hipokampal nöronları bazı hücreler dejenerasyon ve kan damarı blokajları gösterırken diğer gruplarda (A,C,D) bu belirtilere rastlanmadı. Cresyl violet ile boyanma göre B grubunda en az yoğun olarak bulundu. D grubundaki denekler eşit miktarda 3-NP aldıklar halde bunların preparatlarında B grubundakilerde görüldüğü üzere benzer nöral dejenerasyon izlerine rastlanmadı. Bu çalışma sonunda Garcinia kola’nın kötü beslenen farelerin serebellum ve hipokampuslarındaki nöronlar üzerinde koruyucu bir etkisi olduğunu kanısına varıldı.

Anahtar Kelimeler: Garcinia kola, 3-Nitropropionik asid, Nöron, Hipokampüs, Kötü beslenen fare, Serebellum

INTRODUCTION

Garcinia kola (Bitter kola) and its relatives including Garcinia livingstonei, Garcinia gnetoides, Garcinia staudtii, Garcinia smeathemannii, Garcinia ovalivolia, Garcinia brevipediellata and Garcinia mannii are found in Nigeria as well as across the humid lowland plain of West Africa extending from Sierra Leone to Zaire (22,28).

Garcinia kola seeds are chewed as a masticatory substance to stimulate the flow of saliva, and widely consumed as snack(3). 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(3).

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(9).

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 in Garcinia kola, seeds which are widely consumed as a stimulant(24). The traditional African medicinal uses include treatment of cough, purgative, anti-parasitic and antimicrobial(30,34). The seed is used in the treatment of diarrhoea(11,12) bronchitis and throat infections(2,36) and liver disorder(26). The Garcinia kola seeds enjoy a folk reputation in Africa as a poison antidote(5). In addition, the plant possesses anti-hepatotoxic(7,12), antioxidant (35), hypoglycemia(4,26) and aphrodisiac. properties(6).

Garcinia kola seeds have been reported to have an anti-inflammatory activity(13,30). These studies have revealed that the process of ovulation is comparable to an inflammatory process(20). Anti-inflammatory drugs have been employed in blocking ovulation(27).

Some flavonoids (including apigenin based) suppress the formation of cyclooxygenase-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(20).
Garcinia kola seeds contain biflavonoid capable of having anti-inflammatory properties\(^{(13)}\) and are a natural antioxidant\(^{(35,38)}\). Constituents of the seed of Garcinia kola include 1 – 3, 8 – 11 benzophenones, Garcinia biflavonones (GB-1, GB- 2) and kola flavonone (Cotterih et al., 1978). Apigenin based flavonoids represent 60% of the total flavonoids present in the diethyl ether fraction of Garcinia kola seed\(^{(25)}\). 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. Saponin for example is haemolytic in nature\(^{(16)}\). Major adverse effects of these secondary plant metabolites are known divalent metal chelators, protein binders, antinutrients, respiratory system and oxidative phosphorylation poisons\(^{(11,18)}\).

The tannin, saponin, oxalate, cyanogenic and cardiac glycosides of Garcinia kola are 0.69 mg/100g, 15.7 mg/100g, 1.707 mg/100g, 59.56 mg/100g and 67.10 mg/100g of dry matter respectively\(^{(16)}\). The essence of estimating the concentrations of these secondary plant metabolites is to establish and advice on the quantity one can chew at a time. The higher the concentration of these metabolites the more dangerous they become to health.

We therefore aim at elucidating pharmacologic importance of Garcinia kola which 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\(^{(14)}\).

**MATERIAL AND METHODS**

**Care and Management of The Animals**

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. Proper care of the animals was in accordance to Leader\(^{(29)}\) and the National Institute of Health (U.S. PHS policy) on humane care of laboratory animal\(^{(31)}\).

**Feed Preparation**

The formulation of the malnourished feed with which the animals were fed was based on the estimations of Robinson and Nwoha (33,37)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount (g)</th>
</tr>
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<tbody>
<tr>
<td>Grains</td>
<td>72</td>
</tr>
<tr>
<td>Casein</td>
<td>3</td>
</tr>
<tr>
<td>Cellulose</td>
<td>8</td>
</tr>
<tr>
<td>Oil</td>
<td>6</td>
</tr>
<tr>
<td>Sucrose</td>
<td>5</td>
</tr>
<tr>
<td>Vitamin</td>
<td>6</td>
</tr>
</tbody>
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Accurate measurements of the parameters stated above was ensured with the use of Mettler Toledo sensitive balance (model no. 153). They were mixed together with water, molded and oven dried at 45°C before feeding the animals with it.

**Extract Preparation**

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.

**Drug Administration**

3–Nitropropioic 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-Nitropropioic 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 and cerebellum\(^{(23)}\).
Excision of Hippocampus

After sacrifice, the skulls were opened up with the aid of a pair of bone forceps to expose the brains which were removed, weighed and fixed in 10% formal calcium immediately. Thereafter, the hippocampus and cerebellum were removed with the aid of a sharp scalpel blade for:

i. Haematoxylin and Eosin staining, and

ii. Demonstration of Nissl substance.

RESULTS

Haematoxylin and Eosin (H&E) sections of hippocampus and cerebellum, showed layers and neuronal cells that was distinct for groups A, C and D. The neuronal cytoplasms were normal. The blood vessels were clear and well placed, without any form of disruption and blockage (Figs.1 and 3) while that of group B showed neurons with cytoplasm that were faintly stained. The blood vessels were occluded. Hippocampal regions and cerebellar area were still clearly seen (Figs.1 and 3).

Groups A, C and D showed intensely stained nissl substances in the neurons of hippocampus and cerebellum. The hippocampal regions and cerebellar area were all intact and the blood vessels were not disrupted. (Figs.2 and 4) while group B showed that the nissl substances of the neurons were less intensely stained. The blood vessels were seen disrupted and atrectic. (Figs.2 and 4).

Figure 1: Sections of Cerebellum (a) group A showing normal blood vessel (B) and intact neurons (N), (b) Group B showing blocked blood vessel (arrow), (c) group C showing a normal blood vessel (arrow), (d) group D showing intact neurons (arrow), H& E X100.
**Figure 2:** Sections of Cerebellum (a) group A with intact neuron (arrow) X400, (b) Group B showing degenerated neuron (arrow) X100, (c) group C showing intact neuron (arrow) X100, (d) group D showing intact neurons (arrow) X100, Nissl stain.

**Figure 3:** Sections of Hippocampus (a) group A showing normal blood vessel (arrow) X40, (b) Group B showing degenerated neuron (arrow) X400, (c) group C showing normal neuron (arrow) X400, (d) group D showing intact neurons (N) and normal blood vessel (B) X40, H&E.
DISCUSSION

The microstructural results which showed neurons with cytoplasm faintly stained in the hippocampus and cerebellum with ruptured and blocked blood vessels for the malnourished mice that were treated with neurotoxins (group B) compared with others i.e. groups A, C and D, were indications for cell death. Cell death are of two types, namely apoptotic and necrotic cell death. These two types differ morphologically and biochemically\(^{(41)}\). Accidental cell death is regarded as necrotic and could result from extrinsic insults to the cell such as osmotic, thermal, toxic and traumatic effects\(^{(17)}\). Pathological cell death is regarded as apoptotic and is an organized programmed cell death (PCD) that is mediated by active and intrinsic mechanism. Therefore, it could be deduced that the cell death noticed in this work was due to the effect of the neurotoxins on the neurons which was ameliorated by the aqueous extract of Garcinia kola given to the malnourished mice orally.

In cellular necrosis, the rate of progression depends on the severity of environmental insults. The greater the severity of insults, the more rapid the progression of neuronal injury. 3-Nitropropionic acid destroys neurons both directly at the level of the cell body and at the axonal domain by showing retrograde degeneration due to damage of both their afferents and their parent axons\(^{(43)}\). The mechanism of striatal cell death after systemic administration of 3-Nitropropionic acid is not well understood, although several factors may contribute to selective vulnerability of medium-sized spiny striatal neurons. Decortication, which removes putative glutamatergic inputs to the caudate putamen, can protect against 3-Nitropropionic acid toxicity in the caudate putamen\(^{(10,19)}\), and N-Methyl-D-Aspartate receptors are up-regulated in both cortex and caudate putamen following 3-Nitropropionic acid injection\(^{(40)}\). These are both lines of evidence for a secondary excitotoxic mechanism, which endogenous glutamate can become toxic following a compromise in cell's energy levels\(^{(8,32)}\).

Epidemiology and dietary intervention studies in humans and animals provide evidence that flavonoid consumption from fruit, vegetables, and plant-derived beverages, for examples, is important to
neuronal health\(^{(42)}\). It was observed in this research work that Garcinia kola which has abundance of flavonoid actually prevented neuronal damage in the malnourished mice.

The CYP1 family is comprised of three known enzymes, CYP1A1, CYP1A2, and CYP1B1\(^{(20)}\). Dietary-based agents from several chemical classes have been found to be inhibitory of their catalytic activities. Most of the studies have examined flavonoids and found a number of them to be potent inhibitors\(^{(21,39)}\). Flavonoids are a diverse group of phytochemicals that are produced by various plants in high quantities\(^{(15)}\).

Nissl substance had been reported to play key roles in cellular metabolism\(^{(1)}\). Its depletion in malnourished mice treated with 3-Nitropropionic acid (group B) was as a result of health hazard at the neuronal level, this was prevented in malnourished mice fed with extract of Garcinia kola then treated with neurotoxin (group D) due to the antioxidative nature of the Garcinia kola extract that prevented the oxidation which 3-Nitropropionic acid would have caused to CYP1 enzymes. Therefore, flavonoids constituent of the Garcinia Kola is performing the role of the potent inhibitor.

The results obtained in this study following administration of 200mg/kg body weight of Garcinia kola extracts for 7 days orally prior to treatment with 20mg/kg body weight of 3-Nitropropionic acid dissolved in distilled water and administered intraperitonially to malnourished mice, showed that neurotoxins caused neuronal degeneration which were prevented by intake of Garcinia kola prior to treatment with 3-Nitropionic acid. The protective property of Garcinia Kola is believed to be due to its antioxidant content which prevented neuronal degeneration and blood vessels damage.

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