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Red Onion Juice (*Allium Cepa Linn*) and Carica Papaya Seed Extracts Ameliorated the Toxic Effects of Chronic Consumption of Cassava Flakes, On Vision.

Nelson Emirene Iyio[1], Bright Ichechi Owhorji[2], Harold Chris-Biriowu[3], Minini Odimabo[4]

[1,2,3,4]Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Rivers State University, Port Harcourt.

ABSTRACT

This study investigated the impact of chronic consumption of cassava flakes on vision, and the ameliorative potential of Carica papaya seed and red onion on these effects. Cassava flakes, widely consumed in Nigeria, contain cyanogenic glycosides that can lead to cyanide toxicity, resulting in visual impairments. Using an experimental approach, forty Swiss white mice (40-50 g) were randomly divided into four groups of ten mice each. The groups were assigned specific treatments: Group 1 served as the control (distilled water) plus rat feeds, Group 2 received normal rat feed and cassava flakes, Group 3 was treated with normal rat feeds plus cassava flakes plus 4mls/kg of red onion extract, Group 4 received normal rat feeds plus cassava flakes plus 4mls/kg of Carica papaya seed extract. Visual acuity test was done using the Visual Water Maze. Results revealed that mice in the Cassava flakes-only group exhibited significant deficits in visual performance, with corresponding neuronal and retinal damage. Supplementation with Carica papaya seed extract and onion extract ameliorated these effects, improving vision and reducing oxidative stress and tissue damage. These findings underscore the adverse effects of chronic consumption of cassava flakes and highlight the neuroprotective and restorative properties of Carica papaya seed extract and onion juice.

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BACKGROUND OF THE STUDY.

Social determinants of health are generally defined as the conditions in the environments where people are born, live, learn, work, and play (Reshetnyak et al 2020). and it has become evident that these social factors affect health outcomes (Reshetnyak et al 2020). Vision health is no exemption (Elam et al 2013). For example, among patients with diabetes, glycaemic control and disease duration account for only 11% of the risk of developing microvascular complications like diabetic retinopathy, suggesting that environmental factors likely play an important role in the disease course (Nwanyanwu et al 2015).

There is a high prevalence of blindness and severe visual impairment especially among those older than 40 years in Nigeria (Kwari et al 2009). It is estimated that more than 4.25 million adults in Nigeria older than 40 have moderate or severe visual impairment or blindness (Mohamed et al 2009).

It was reported that tropical ataxic neuropathy and some similar degenerative neuropathies that may cause poor vision (visual defects) occur in Nigeria mainly amongst older people who might have consumed cyanide mainly from cassava products over many years (Oluwole et al 2004) suggesting the effects of chronic systemic cyanide toxicity. This research study focuses on the impact of casava flakes (Garri). on poor vision, and how cassava onion extract, and carica paya seed phytochemical nutritional constituent can improved vision.

Cassava is a major staple consumed in Nigeria and high intake has been associated with visual impairment in majority of Nigerians (> 40 years) due to high cyanide content and micronutrient deficiencies. (Emmanuel et al 2009). There is a high prevalence of blindness and severe visual impairment especially among those older than 40 years in Nigeria (Kwari et al 2009). Cyanide can increase thiocyanate concentrations

in the body due to chronic cyanide exposure which can adversely affect the thyroid gland. Acute cyanide exposure mainly adversely affects the central nervous system (CNS) and the cardiovascular, endocrine, and respiratory systems (Bolarinwa et al 2016). The toxic effect of cyanide is most pronounced in organs related to the CNS because of the high demand for oxygen by such organs especially the brain, heart and eyes.

In Nigeria, practically every community is heavily dependent on cassava due to its widespread use in Garri, Akpu, Fufu, Chips, and Starch production. Prior to ingestion, cassava often passes through processing as a method for preservation, cleansing, and change brought about by the cyanogenic glycoside present in the roots and unfermented leaves. Because the concentration of cyanide varies throughout cassava species, consuming cyanide through processed cassava flakes (garri), cooked cassava, or raw cassava may potentially have negative effects on consumers.

The study aimed at Investigating the effects of chronic consumption of cassava flakes, onion juice (Allium cepa linn), and carica papaya seed extract on vision in Swiss white mice.

According to a study, (Nyenwoke et al, 2014), cassava is vegetatively propagated through stem cutting and produces well on poor soils. He added that the tubers may be kept in the soil for extended time periods. This features and high crop yield contribute to the importance of cassava in Africa, Southeast Asia and South America. Also, cassava can be grown on poor soil with little or no investment in irrigation, fertilizer or pesticides. Schael, (1999), studied the history of the crop and stated that the beginning of interest in the crop in recent time results from the realization of the potential of cassava as a food security and emergence crop which could generate employment for the rural poor and foreign exchange for the country. Since 1990, the

government, through the ministry of food and agriculture has demonstrated its determination and commitment to promote cassava for the alleviation of poverty particularly in rural households and communities. He maintained that the main reasons for expansion of cassava production are population growth, famine or seasonal hunger and market availability. In Nigeria, cassava is moving from starvation prevention crop to cash crop for local urban consumption. The top cassava producers are in order, Nigeria, Brazil, Thailand and Indonesia. The next six countries are all in Africa.

Materials & methods:

The experiment was carried out in the animal house of Faculty of Basic Medical Sciences, University of Port Harcourt. A total of forty (40) mice weighing 40-50g was obtained from the experimental animal unit of Department of human physiology, University of Port Harcourt, Rivers State. The mice were kept in clean disinfected wooden cages with saw dust as beddings in the animal house, with 12hours light/dark cycle and 50-60% humidity at a temperature of about 30^{o}C and were allowed to acclimatize to the new environment for two weeks, with free access to clean water and animal feed. The mice were be weighed using an analytical weighing balance at commencement of the experiment. Cassava flakes, onions and pawpaw were bought from the local market at Mile 3, A certified botanist from the Department of Plant Science and Biotechnology, University of Port Harcourt verified the botanical identification of these plant materials to ensure accuracy (UPH PSB H126, UPH PSB 127, UPH PSB 128). The onion was peeled, washed, and chopped into small bits. They were air-dried and grounded into fine powder. This powder was made to undergo a solvent extraction process utilizing 95% of ethanol. After soaking for 48 hours, the mixture was filtered using a sieve, and the resulting liquid extract was concentrated by evaporation to produce a concentrated onion extract. Forty mice were randomly divided into four groups. Group 1 (control) received rat feeds, Group 2 received normal rat feed and cassava flakes. Group 3 was treated with normal rat feeds plus cassava flakes plus 4mls/kg of red onion extract, Group 4 received normal rat feeds plus cassava flakes plus 4mls/kg of Carica papaya seed extract. Visual acuity test was done using the Visual Water Maze.

GROUPS	Treatment
GROUP 1 Control (10 rats)	Rat feeds
GROUP 2 (10 rat)	Normal rat feeds + Cassava flakes (30:70)
GROUP 3 (10 rats)	Normal rat feed + Cassava flakes (70:30) + 4ml/kg of onion extract)
GROUP 4 (10rats)	Normal rat feed + Cassava flakes (70:30) + 4ml/kg Carica papaya extracts.

Visual Water Maze: The Visual Water is a trapezoidal-shaped pool 30cm deep, with sides measuring 10cm by 40cm by 90cm. The narrow 10cm end is the entry point for each mouse. The wider end 40cm of the pool is divided into two decision zones. High-resolution monitors are placed in the decision zones at the end of the maze's "arms". The screen where the hidden escape platform was placed displayed visual pattern of a moving grating while the other screen displayed only a static white screen. The mice were taught to associate the moving grating with the escape platform.

Initial Training Phase: The mice were placed into the water at the starting narrow end and was given a limited amount of time (30 seconds) to find the hidden platform. If the mouse did not find it after 30 seconds, it was guided to the platform

and allowed to stay on top for another 30 seconds. Over successive trials, the animal learns to use the moving grating as a cue to find the platform more quickly. Each animal had three trials each day for 2 days. At the end of each trial, they were allowed to rest in a heated enclosure. After each trial, the escape platform was moved to the opposite zone but with the moving grating monitor.

Testing Phase: On the third day, the mice were introduced into the maze to swim towards the platform side to locate the hidden platform. The platform was kept in a constant location with the monitor with the moving grating. The time taken to find the platform (latency) was recorded. The shorter the time to find the platform, the better their vision.

Novel Object Recognition Test (NORT) is a behavioural paradigm used to assess recognition memory in animals, particularly rodents. It is based on the idea that animals have a natural tendency to explore new objects more than familiar ones, and this preference can be used to gauge their ability to recognize and remember objects. Familiarization Phase: In this phase, the mice is placed in a test arena where it encounters two identical objects. The animal is allowed to explore these objects freely for a one minute so it can become familiar with both objects.

Testing Phase: The mice is returned to the arena, but one of the previously encountered objects is replaced with a novel (new) object. The animal is allowed to explore the objects for a set three minutes. The time the mice spent exploring each object (familiar vs. novel) is taken.

The data gotten was analysed using the Statistical package for social sciences (SPSS) software version 20. Statistical significance of the differences between the groups was be determined by Analysis of Variance (ANOVA) and Turkey post Hoc test.

RESULTS:

Table 1. Result of Visual Water Maze.

GROPS	Day 1 (Sec)	Day 2 (Sec)	Day 3 (Sec)
Group 1	47.80±8.3	32.20±1.6	23.80±3.3[a]
Group 2	48.60±8.7	113.60±3.7	173.00±6.3
Group 3	45.40±9.9	36.20±3.5	23.80±1.8[a]
Group 4	43.60±8.3	37.40±8.6	21.30±7.4[a]

Values are presented in mean ± SEM. n= 5. [a] Means values are statistically significant ($p<0.05$) when compared to the group 2.

The results from Table 1 indicate that the group 4 exhibited the best performance in the Visual Water Maze, with the shortest time on the test day, suggesting strong visual perception. Group 3 which combines cassava flakes and onion extract, had better performance across day 2 and 3.

Table 2. Result of Novel Object Recognition test

	Week 1		Week 2		Week 3	
	old object	novel object	old object	novel object	old object	novel object
1	59.20±0.8	43.70±1.5	41.42±25.2[a]	24.22±5.7	21.20±3.7	61.20±2.4
2	58.01±4.3	13.00±5.7	58.50±4.8	23.10±7.7	51.20±5.4	21.00±4.4
3	58.01±4.3	13.00±5.7	58.50±4.8	23.10±7.7	51.20±5.4	21.00±4.4
4	66.00±6.6	23.35±5.3	34.57±7.7	43.62±5.6[a]	63.21±4.5	71.32±4.7[a]

Values are presented in mean ± SEM, n= 5. [a] Means values are statistically significant ($p<0.05$) when compared to the control group 2.

Table 2 presents the outcomes of the Visual Acuity using the novel object recognition test across different treatment groups over three weeks. The data shows that by the third week he Group 4 demonstrated a significantly longer exploration time with the novel object 71.32±4.7 seconds.

DISCUSSION

Results of Visual Acuity conducted in the different treatment groups provide important information on the impact of the different diets on vision, as measured by visual Water Maze and Novel Object Recognition tests. In the Visual Water Maze, the Normal Control group exhibited optimal performance indicative of good visual perception. These findings correspond with the literature indicating that a healthy physiological status, characterized by optimal neurotransmitter levels and neurotrophic factor expression, contributes positively to cognitive functions (Teleanu et al., 2022). On the other hand, Groups 3 and 4 had a good performance comparable to the control group. Group 2 that received cassava flakes only demonstrated the worst visual acuity. This has somewhat corroborated by other studies showing that cassava diet may worsen vision due to high cyanide content. Carica papaya is reported to have antioxidant activity. This may have influenced the positive impact on the visual acuity. This hypothesis is further corroborated by the erratic performance of Group 2, which consisted only of Garri, as carbohydrate-heavy diets like cassava may affect cognitive and visual health in a negative way in the brain (Walk et al., 2017). The Novel object recognition (NOR) test takes advantage of mice's natural

propensity to investigate unfamiliar objects more thoroughly than ones they are familiar with. And of course, without proper visual acuity, this won't be possible. Researchers can determine visual processing by comparing the amount of time spent investigating new vs familiar objects. After a retention interval, one object is replaced with a novel object, and the amount of time spent with each object is measured. Increased exploration of the novel object indicates successful memory encoding and retrieval which is somehow dependent on good vision. The performance seen in Group 1 showed intact vision. In contrast, group 2 showed poor visual performance. Similarly, the pattern of results for Group 3, onion extract, suggests remarkable visual function; this result supports evidence present in the literature that onion exerts neuroprotection because of its flavonoid content. They point out that dietary components and their interactive effects result in a much more nuanced role in vision and cognitive performance, specifically cholinergic activity and neurotrophic support. These findings underline the critical need for further research to identify specific mechanisms of action whereby these agents modulate vision, learning and memory for overall cognitive health. The group that received only Carica papaya seed extract (Group 4) developed reasonable visual recognition performance in comparison; this might have been due to the fact that Carica papaya contains a broad spectrum of phytochemicals including enzymes (in the latex), Carotenoids (in fruits and seeds), alkaloids (in leaves), phenolics (in fruits, leaves, and shoots), glucosinolate (in seeds and fruits) (Krishna et al., 2008; Parle et al., 2011). Some important phytochemicals found in C. papaya are Lycopene, Betacarotinoid, Benzyl isothiocyanate, Betacryptoxanthin, Benzylglucosinolate, chlorogenic acid, caffeic acid, protocatechuic acid, Quercetin etc. certain studies indicate that a diet including a high intake of simple carbohydrates contributes to insulin resistance and fluctuations in blood sugar levels that could impair visual function. This also agrees with the view expressed in some studies which concluded that high carbohydrate diets can negate the protective effects of dietary antioxidants, thus indicating a need to have a balanced diet as part of the strategy for cognitive health.

Conclusion:

The outcome showed that mice fed with cassava flake developed significant impairments in visual recognition, as reflected by poorer performances in both the visual water maze and the Novel Object Recognition test. However, administration of onion juice and carica papaya improved it.

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