

Neurobehavioural Changes and not Structural Changes Occur in Rat Fed with Cassava Products (Fufu, Garri and Tapioca Diet)

ABSTRACT

The ability of cassava products (fufu, garri and tapioca) to produce behavioral and structural changes in the brain of adult rats was examined. Forty adult male rats (N=40) were used for this study and the experiment was divided into two phases (N=20 each). Rats were trained in a Reach-To-Grasp Task for 6 weeks and Quantitative assessments using a reaching movement scale and reach-to-grasp success rate were carried out to determine baseline values. After which administration of the various cassava products was done. The 1st phase had the rats trained to perform the reach to grasp experiment before being fed with various cassava diet. In this phase the animals performed significantly worse than the controls when determining the average success rate in a reach-to-grasp experiment (baseline=72.6%, fufu diet= 30%, garri diet =47%, tapioca diet =59%). The 2nd phase had the rats trained simultaneously as they were being fed with various cassava diet. In this phase the animals also performed significantly worse than the controls when determining the average success rate in a reach-to-grasp experiment (fufu diet= 37.5%, garri diet =46.5%, tapioca diet =56.5%). The various movement of the rat was assessed using Tracker software (Video Analysis and Modeling tool, version 5.1.2) and the movement patterns were determined. The animals fed with Fufu shows the worst movement pattern indicating aiming impairment which may be as a result of structural changes in the m1 region of the cerebral cortex. The data obtained were analysed using ANOVA and there was significant difference ($p < 0.05$) in the movement pattern. Histological observation showed no neuropathological changes in the motor cortex. The rats fed with cassava diets shows no observable changes in the structures and sizes of the neurons as compared with the control. Cassava products (Fufu, Garri and Tapioca) have been demonstrated to cause neurobehavioural and mild structural changes in the M1 region of the cerebral cortex.

Key Words: Cassava, Garri, Tapioca, Motor cortex, Neurobehaviour, Neurons,

1. INTRODUCTION

Cassava and its various products (fufu, garri, tapioca) are a very rich source of carbohydrate compared to other carbohydrate-containing foods (e.g. rice and maize). Cassava however, contains cyanogenic glycosides which release hydrogen cyanide when chewed or digested. Due to hydrolysis cyanide is released when cassava is chewed or digested and this has the ability to cause acute cyanide poisoning when it is consumed continuously. Cyanide toxicity causes an irreversible neurological condition in the long term such as Konzo and Tropical Ataxic neuropathy (TAN) (Enefe *et al.*, 2020 and David *et al.*, 2021). According to Boivin *et al.* (2013), cassava cyanide poisoning was shown to be associated with cognitive deficits and severe impairments of fine motor control and coordination using the Kaufman Assessment Battery for Children, 2nd edition (KABC-II) for cognition and the Bruininks/Oseretsky Test, 2nd Edition (BOT-2) measure for motor proficiency. Motor symptoms of konzo are permanent and irreversible. Deficits in fine motor control, in association with exaggerated deep tendon reflexes or ankle clonus, are commonly found in the general population of konzo-affected areas, suggesting the existence of subclinical and preclinical konzo (Howlett *et al.*, 1990; Tshala-Katumbay *et al.*, 2001; Cliff *et al.*, 1999; Al-Kuraishy *et al.*, 2016; 2017; Waheed *et al.*, 2019; 2020). The World Health Organization (1996), graded the severity of konzo according to the following: mild konzo (able to walk with no support), moderate konzo (needs support to walk), and severe konzo (Unable to walk).

Tracy and Wishaw, (2002) explained the effect of a lesion in the motor cortex; the mice had higher scores before the lesion with an average percent success of 40 ± 10 before the lesion and 10 ± 10 average success percent after the lesion. This showed that the performance of the lesion group was inferior to that of the control group on all the test days.

These products of cassava that were not properly processed have the tendency to cause neurobehavioral effects on consumption. The extent of the damages is not all known yet, but considering the presence of cyanide in those products and at what quantity; there are anticipated neurobehavioural changes in the rats fed with these products which can also happen in humans. Hence, this study was done to demonstrate the ability of cassava products (fufu, garri and tapioca) to produce behavioral changes in the brain of adult rats.

2. MATERIALS AND METHODS

Forty Male Wistar rat were used for this experiment and they were kept to acclimatize to their new environment for about 2 weeks. The animals were kept singly in their cages (Single-housing

system) and were food deprived according to their body weight so as to motivate the animals to perform the reach-to-grasp task. This single housing was to ensure that the right quantity of food is given to each animal, thus maintaining sufficient motivation and optimal reaching performance. The rats were divided into 2 phases and 4 groups in each phase: the first phase was trained first using the reaching apparatus before fed with the cassava diets while the second phase was fed with the cassava diets from the onset. Each phase was divided into 4 groups, each fed with the various cassava diets (fufu, garri, tapioca) and the control. After the acclimatization process the rats were trained to perform the reaching task for 6 weeks and the baseline scores were achieved. The rats were then fed with the cassava diets.

2.1 Cassava Preparation for Consumption by Rats

Fresh cassava products (fufu, garri, Tapioca) were obtained and were administered to the rats for about 6 weeks.

Fufu: In making Fufu, the cassava tubers were harvested and peeled, washed properly and soaked for 3-4 days to properly ferment. It was then filtered with the addition of water to get a required mash. The mash was placed in a bag and pressed to drain out excess water, large moulds are made and placed in a boiling pot to simmer for a few minutes. The moulds were then pounded properly and made into smaller moulds which are then wrapped, stored and given to animal when needed.

Garri: In the making of garri flour, cassava tubers are peeled, washed and grated or crushed to produce a mash. The mash was placed in a porous bag, which is then placed in an adjustable press machine for 1–3 hours for the removal of excess water containing cyanide. The mash is then left to ferment and properly dry up for a few days, once dried it is then sieved and fried in a large pot. The resulting dry granular garri was stored for the duration of the experiment. The garri diet was then prepared and given to the animal.

Tapioca: Cassava tubers were harvested and peeled. They were washed properly and soaked for about 3-4 days. The peeled cassava tubers were then cut in slices and boiled properly. The soft slices were soaked in water to prevent them from drying up.

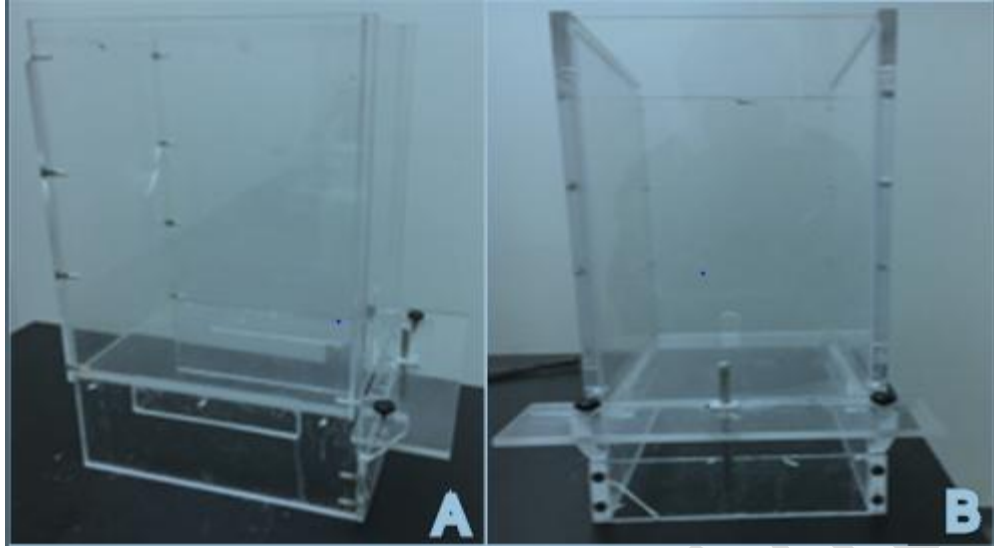
2.2 Behavioural Assessment

The animals were trained to perform the reach to grasp task in which the baseline was achieved and subsequent success rates were also gotten from animals fed with cassava diets across the groups. The pellets were placed on a pedestal for the animals to attempt to reach and grasp. Their pattern of limb movement and process of acquiring the pellets were assessed. Before the training the rats were placed on a food restriction regimen, this was to get down their weight to about 70% so as to motivate them to perform the training. At the start of the restrictions, animal's body weight was gotten. Then, the food was restricted to 70% needed to maintain the body weight. The 70% value was recommended by both Institutional Animal Care and Use Committee (IACUC) documents consulted of Emory University of Pennsylvania Philadelphia, USA. This guideline was used; to every 1kg body weight 86g of food was given (USEPA, 1988).

The reaches of each rat was recorded as follows: When the rats successfully brought the pellets to the mouth it was recorded as a "success". When the rat successfully brought the pellet to its mouth after several reaches it was recorded as a "Success" but not as a "1st Attempt". If it dropped along the way of getting to the mouth it was recorded as a "drop". Those that were knocked off from the stage were recorded as a "fail". The success, drop and failed scores were then analyzed to give their percentages. A video recording was also carried out in each section for off-site analysis. The dexterity or handedness of the rats was also noted in the trial process. Before each training session, the weight of the animals was checked first to determine the current weight and observe if there was a loss or gain in weight.

2.3 Apparatus

The reaching apparatus has the following dimensions (Tracy and Han, 2002) :Height 20cm, Length 19.5cm, Breadth 8cm, Slot 1cm, Slot to stage 3cm. The rats were trained in the reaching apparatus; a transparent box (made with plexiglass), with a 1cm wide slot through which they reach and grasp flavored pellets (Cocopops) from a stage about 3cm from the slot. A trial section began when the pellet was placed on the stage until it was successfully received, dropped or knocked off. A maximum of 50 trials was done each day. Animals reach through a vertical slit at the front of the cage. The mechanisms of a rat's reach are such that, targets contra lateral to the reaching limb are more easily obtained than ipsilateral targets. Thus by placing food to one side or the other, we could elicit reaches from either forelimb, in a controlled manner. The protocol of Wishaw and Pellis (1990) and Ballerman et al., (2001) with minor modifications was followed.



(A)Side View (B)Front View

Fig.1: Reaching Apparatus

2.4 Tissue preparation

The animals were anaesthetized in desiccators using chloroform and then euthanized. Trans cardiac perfusion was performed to fix the brain. The head was removed using a pair of bone crusher. The remaining muscles were removed to expose the skull. The brain of the animals was harvested and was enclosed in test-tube containing formalin to fix properly before tissue processing. Coronal sections were taken on the cerebrum and cerebellum using a microtome at 5 μ .

2.5 Staining Procedures using Toluidine Blue

The slides were deparaffinized in xylene for 1 minute and then immersed in different grades of alcohol (90% and 70%), before being rinsed in water. The slides were immersed in sections to distilled water. The sections were put in toluidine blue stain for 2-3 minutes till the color of the section became blue. Each slide was washed in distilled water for 3 changes and then dehydrated quickly through 95% and 2 changes of 100% alcohol (10 dips each since stain fades quickly in alcohol). Clearing in xylene or xylene substitute was done for 2 changes at 3 minutes each. A

cover slip with are sinous mounting medium were used to cover the sections while preventing air bubbles from getting in.

2.6 Statistical Analysis

Graph prism 7, was the software used to analyze the weight and success rates by plotting them on a graph. ANOVA and Bartlett's test of Homogeneity were used as a parameter for data analysis; this was done on a graph that showed the progression of success rates. Rat movements and trajectory were analyzed using tracker software (Video Analysis and Modeling tool, Douglas Brown 2020 version 5.1.2) and the velocity of movement of the rat was calculated.

3. RESULTS

3.1. Weight Assessment of Animals

Statistical analysis was carried out using ANOVA to determine the differences in the weight between the various groups (baseline, fufu, garri, tapioca). Differences between-group variances were assessed with Bartlett's test of Homogeneity, with $p < 0.05$ set as the significance level. Using ANOVA, Significant difference was observed at $P < 0.05$ ($f=29.29$; $p < 0.0001$) across the weight of the groups (Baseline, Garri, Fufu, Tapioca) for phase 1 and at $P < 0.05$ ($f=10.04$; $p < 0.0001$) across the weight of the groups for phase 2. Bartlett's test of Homogeneity of Variances was used to compare variances across weight of the groups. The Bartlett's test statistic was 2.455 at a P value of 0.4834 in the weight of the groups for phase 1. The weight of the groups across phase 2 had a Bartlett's test statistic of 1.479 at a P value of 0.4774.

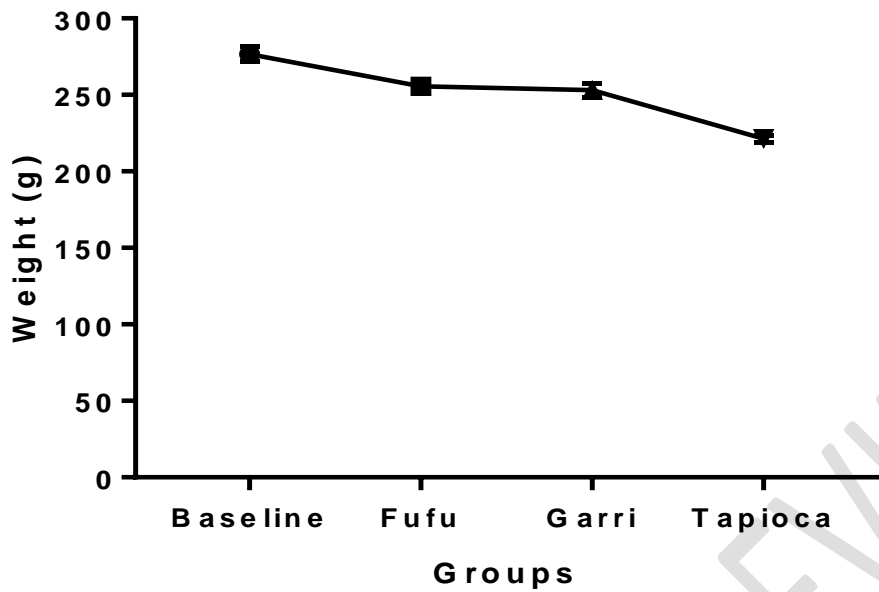


Fig.2: weight of animals at phase1

The weight of the animals was obtained during the reach to grasp experiment and administration of the cassava products on the rats to observe its effects on the weight across the groups. The baseline group had the highest weight after which there was a progressive decline of the weight across the groups.

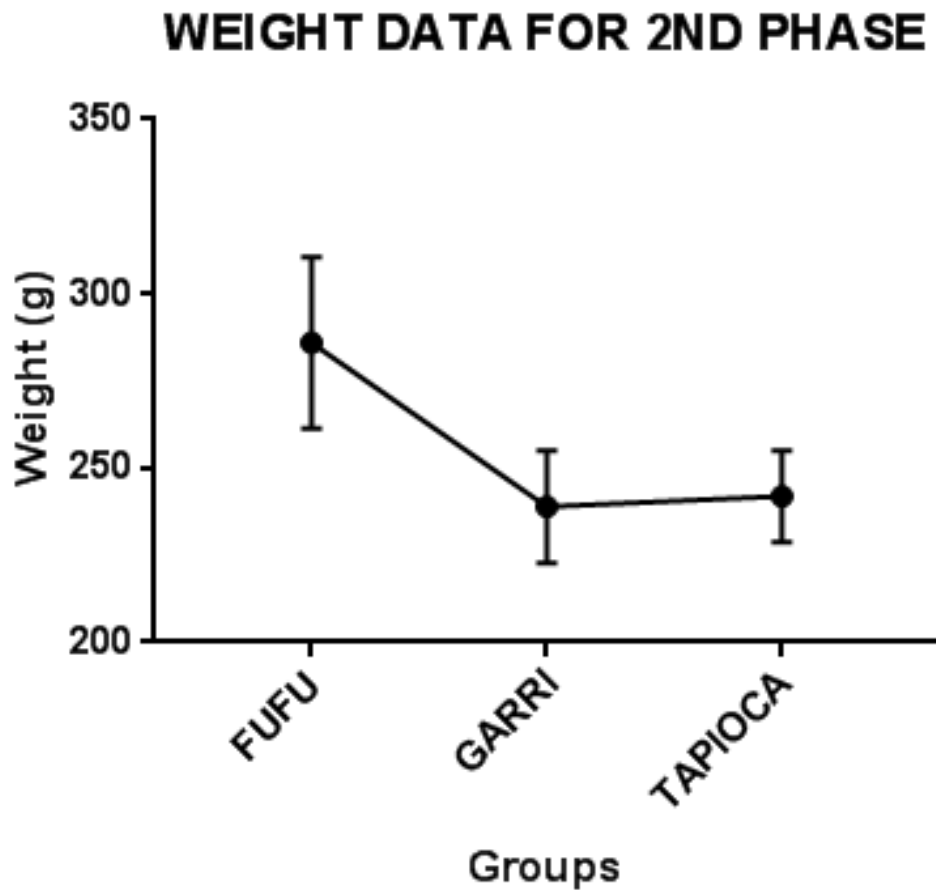


Fig. 3:weightofanimalsatphase2

The weight of the animals was obtained during the reach to grasp experiment and administration of the cassava products; it was observed that the group fed with fufu had the highest weight while those fed with garri showed the most reduction in weight for phase two (fig. 3), but the same way in phase one (fig.2) as Tapioca diet shows much weight reduction.

3.2. Effect of Cassava Diet on Reaching Performance

In the statistical analysis of the success rates across the groups using ANOVA, significant difference was observed at $P < 0.05$ ($f = 11.8$; $p < 0.0001$) across the success rate of the groups (Baseline, Garri, Fufu, Tapioca) for phase 1 and at $P < 0.05$ ($f = 1.456$; $p < 0.0001$) across the success rate of the groups for phase 2. Using Bartlett's test of Homogeneity, the variances across

the success rates of the groups was compared for phase 1 and 2. Phase 1 had a Bartlett's test statistic of 2.067 at a P value of 0.5586 and this showed equal variance across the groups. Phase 2 had a Bartlett's test statistic of 0.3468 at a P value of 0.8408

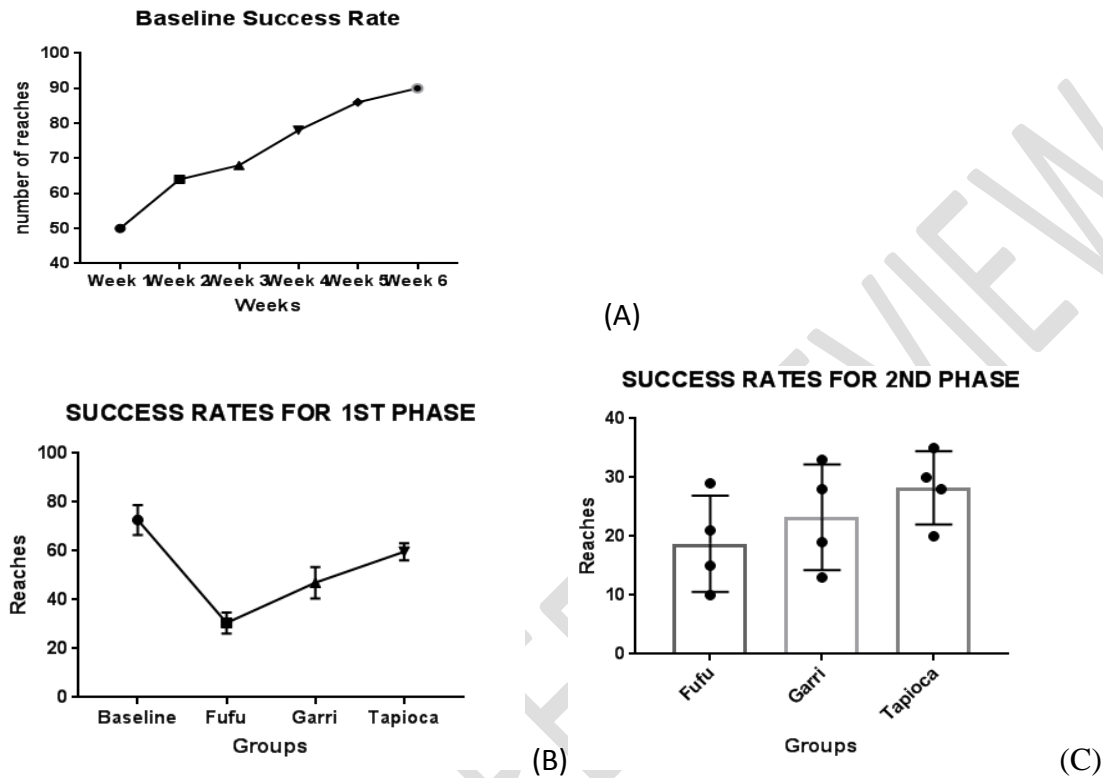


Fig. 4: Success rate Scores (a) Baseline (b) Phase 1 (c) Phase 2

The rats were trained to perform the reach to grasp experiment for 6 weeks. At baseline the success rate was progressive with a steady increase. The rats were fed with the various cassava diets for four weeks. The rats feeding on cassava diets never attained the number of reaches at baseline which they did prior to being fed cassava diets.

3.3. Histological Changes observed in Rats fed with cassava diets

Histological Changes observed in the motor cortex

The control micrographs (Fig. 5a) show numerous neurons with the nucleus closely packed and free of vacuolations in the cerebral motor cortex of the brain. The rats fed with cassava diets

shows no observable changes in the structures and sizes of the neurons as compared with the control (Fig.5b, c, d).

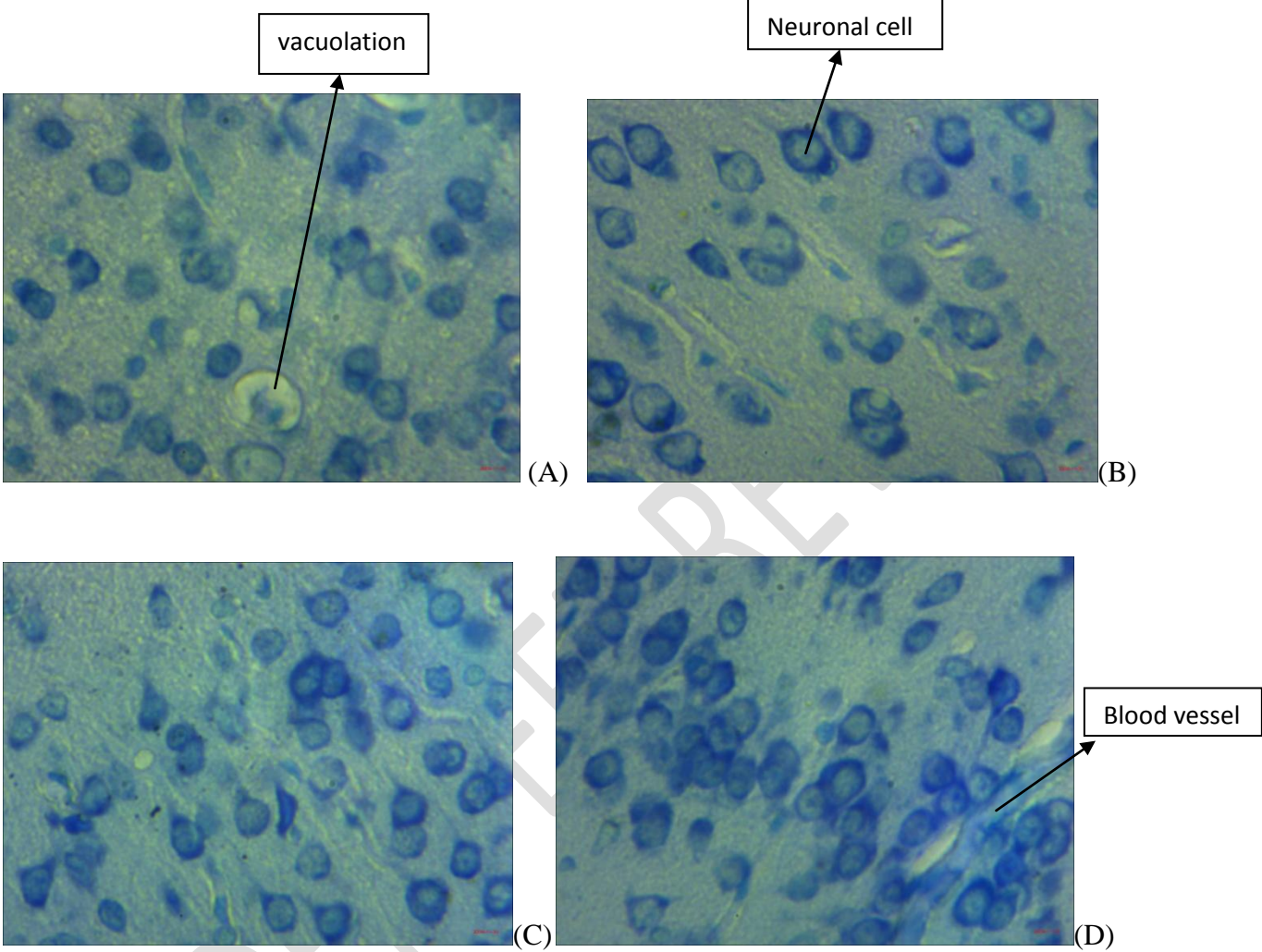


Fig. 5: Photomicrograph of motor cortex Toluidine blue stain: (A) Baseline (B) Fufu (C) Garri (D) Tapioca.

Histological Changes observed in the cerebellar cortex

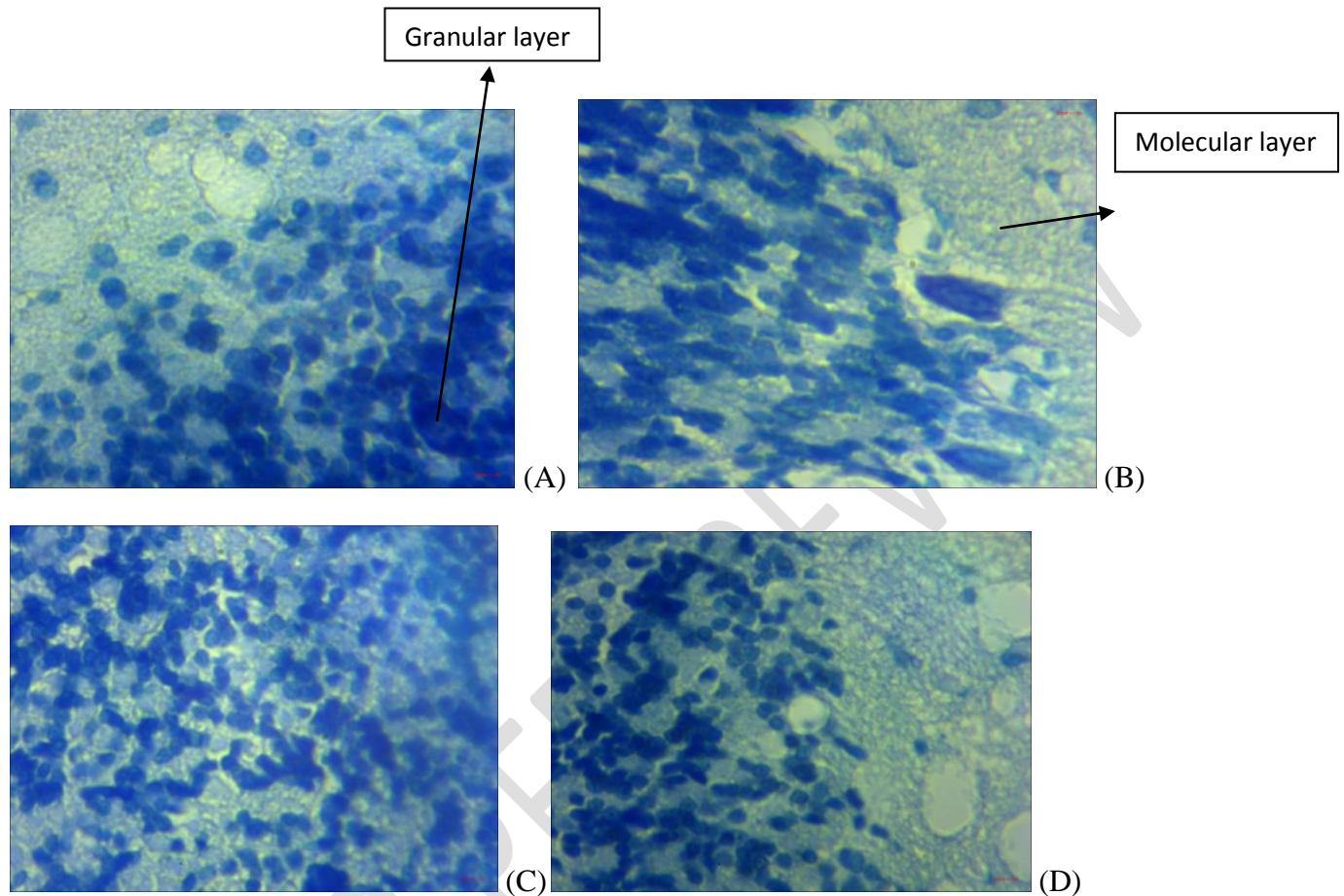


Fig. 6: Photomicrograph of Cerebellum Toluidine blue stain: (A) Baseline (B) Tapioca (C) Fufu (D) Garri

DISCUSSION

The experiment was carried out to examine the effects of the various cassava products (Fufu, Garri and Tapioca) on the rats that fed on them as food. Neurobehavioural changes were assessed quantitative and qualitative. In the experiment, the animals were subjected to a reach-to grasp training process in which they were taught to reach out of the reaching apparatus for pellets (coco pops). During the training process their baseline success rate were recorded and scored.

In the 1st phase of the experiment in which animals were trained before being fed with cassava products, the weights of the animals were determined (fig. 2) and a reduction in weight was observed in all the groups with the group given tapioca having the least weight. The success rate

of the phase 1 rats (fig. 4B) were also gotten and compared to the control success rate (fig. 4A) in which it was observed that the baseline (control group) had an average success rate of 72.6%, the group fed with Fufu had 30% success rate, Garri had 47% success rate and Tapioca had 59% success rate. In the 2nd phase of the experiment, feeding of the animal with cassava product was done simultaneously with the training. The animals took a longer time performing the reach to grasp training as compared to the animals used in the 1st phase where feeding was done after attaining a baseline score for 6 weeks. The weight of the 2nd phase animals (fig 3) were also determined. It was observed that there was a decline as compared to the control weight and in this phase the group given Garri had the least weight and the group fed with Fufu had the most weight. In the comparison of the success rate in this phase (fig 4C) with the baseline (control group) which had an average success rate of 72.6%, the group fed with Fufu had 37.5% success rate, the group given Garri had 46.5% success rate and the group given Tapioca had 56.5% success rate. It took a longer time to train the animals to perform the reach to grasp experiment during this phase. These varying scores in weight and success rate clearly explain that the animals fed with the cassava products (Fufu, Garri and Tapioca) were greatly affected by the toxic effect of the cassava products which restricted them from successfully reaching for the pellets. In both phases it was observed that the group fed with Fufu were the most affected in performing the reach to grasp experiment.

The present study showed that rats' reached for food targets when properly motivated and with the aid of their sense of olfaction. Similar observation was reported by Hermer-Vaquez et al. (2007); Al-Kuraishy et al. (2012); Al-Kuraishy et al. (2017). Animals fed on low protein diet usually stopped growing, lose weight, and sometimes adapt to the diet at a slow rate (Dollet et al. 1985; Okitolonda et al. 1988; Al-Kuraishy et al., 2012; Al-Nimer et al., 2012). Our study agreed with this statement because cassava is low in protein and we observed weight drop in the rats fed with cassava only during our experiment. According to Farr and Whishaw (2002), animals had higher reaching scores before lesion occurred. This was observed in our study as the reaching scores of the animal after cassava consumption decreased when compared with the baseline scores.

This is an indication that bitter cassava may have damaged the motor neuron in the corticospinal tract, hence, affecting the performance of the animals. Previous studies have demonstrated that after a motor cortex injury, a rat still retrieved food by reaching out with the affected forelimb,

but the success rate was reduced (Kolb et al. 2000; Whishaw 2000; Al-Kuraishy et al., 2012; Jasim et al., 2019). They also observed that most of the movement components of the reach are abnormal and those compensatory body movements provided the rotator movements to assist pronation and supination. The finding that rat fed with cassava diets produced a motor impairment is consistent with findings in animal konzo model (Enefa, et. al., 2020 & David, et al., 2021) and human konzo patients (Tylleskar et al. 1993).

Conclusion

This study has shown that the continuous consumption of the cassava products (Fufu, Garri, and Tapioca) causes neurobehavioural changes on the motor cortex which correlates to the disruption of skilled movements in rats. The physical impairments observed on the rats showed the need for the consumption of balanced diet rich in essential protein (amino acids) in humans because the study can also be extrapolated to humans.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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