Acta Scientific Neurology

Volume 2 Issue 7 July 2019

Research Article

Neurotoxicity of Aqueous Extract of *Theobroma cacao* on the Cerebellum of Adult Wistar Rats

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Received: June 13, 2019; **Published:** June 28, 2019

Abstract

This study was aimed at determining the neurotoxicity of *Theobroma cacao* on the cerebellum of adult wistar rats. Twenty four (24) adult wistar rats weighing about 100-160g were used for this research work and were divided into three (3) groups of eight (8) animals each. Group A; control, Group B; low dose and Group C; high dose with eight (8) animals in each group. Control group received vital feed and water ad libitum; the low dose group was administered 240mg kg-1 body weight of *Theobroma cacao* extract and the high dose group was administered 500mg kg-1 body weight of the test substance. All extract was given daily by oral gavage method for twenty one (21) days. Twenty-four hours after the last administration, all animals from each group were sacrificed under chloroform anesthesia. The cerebellum were harvested, weighed and fixed in 10% buffered formalin. Results showed that following administration of extract of *Theobroma cacao* , an insignificant increase in organ weight was observed. Histological observation showed that glial cells, granular cells, medullar of white matter and molecular cell layer appeared normal; no pathology was observed. Study revealed that administration extract of *Theobroma cacao* at these concentrations, showed no neurotoxic effect.

Keywords: *Theobroma cacao;* Wistar Rats; Cerebellum

Introduction

Oral consumption of many natural products (such as plant, leaf, stem, bark) may or may not trigger deleterious responses in many organs of the body at cellular levels [1].

Theobroma cacao is one of the most successful sources of potential drug leads [2]. Its powder has medicinal importance because it has been investigated nutraceutical and traditional medicine. Some part of Africa uses cocoa powder as remedy for managing bronchial asthma, antidiabetic, cardioprotective, anti-hypertensive and anti-malaria agents [3].

Theobroma cacao contains chemicals that increase blood supply to the brain, the chemical composition has also been investigated that *Theobroma cacao* powder contains about 1.9% theobromine, 0.21% caffeine and other related chemicals which if taken much can lead to caffeine-related side effect such a nervousness, sleepiness and a fast heartbeat. Also, *Theobroma cacao* can cause allergic skin reaction, constipation and might trigger migraine headaches [4].

The cerebellum ("little brain") is a structure that is located at the back of the brain, underlying the occipital and temporal lobes of the cerebral cortex. Although the cerebellum accounts for approximately 10% of the brain's volume, it contains over 50% of the total number of neurons in the brain. Historically, the cerebellum has been considered a motor structure, because cerebellar damage leads to impairments in motor control and posture and because the majority of the cerebellum's outputs are to parts of the motor system. Motor commands are not initiated in the cerebellum; rather, the cerebellum modifies the motor commands of the descending pathways to make movements more adaptive and accurate. The cerebellum is involved in Maintenance of balance and posture, Coordination of voluntary movements, Motor learning, Cognitive functions and vision.

Higher plants as sources of medicinal compounds continue to play a dominant role in the maintenance of human health since antiquities. In spite of the huge benefits derived over the years from the use of these medicinal plants, substantial research has shown

the risk involved in the application of some of these plants due to lack of proper dosing, method of preparation and duration of usage (Lev, 2006). Therefore it is important to know the neurotoxicity of *Theobroma cacao* on the cerebellum.

Materials and Method

Extract preparation

Cocoa nut (Theobroma Cocoa) were harvested from a cocoa farm located in Orimkpang Emeh, Boki Local Government Area of Cross River State, Nigeria. The nut were verified and authenticated by Mr. Okon of the Herbarium unity of botany department, university of Calabar. The nuts were plucked, washed to remove debris and air dried at a room temperature of about 27oc for three weeks. They were blended to powder, using a local mortar and pestle. The blended sample of *Theobroma cacao* (cocoa nut) powder was weighted using digital weighting balance and was found to weight 250g.The aqueous extract of the cocoa nut was done using water bath extractor. The extract so obtained was stored in the refrigerator for preservation.

Experimental animals

Twenty four adult Wistar rats weighting about 100-160g were used for this research work. They were purchased from the animal house of the Department of Human Anatomy Faculty of Basic Medical Sciences, Cross River University of Technology (CRUTECH) Okuku and were housed in cages made of wire gauze in the animal house of the Department and were acclimatized in their various cages for a period of two weeks before commencement of the treatment; the animals were housed under standard condition with 12 hours light and 12 hours dark cycle throughout the duration of the experiment.

Experimental design and Procedure

The twenty-four (24) animals were allotted to three groups consisting eight rats each, animals in group A served as the control group, fed with vital feed and distilled water, while groups B and C served as the experimental groups treated with *Theobroma cacao* seed extract, orally for 21days. Group B (Low dose group) animals were treated with 240mgkg-1 body weight of *Theobroma cacao* seed extract, while group C (High dose group) animals were treated with 500mgkg-1 body weight of the seed extract.

Termination of experiment

At the end of the experiment, all animals in each group were sacrificed a day after the end of the last administration of extract under chloroform anesthesia. The cerebellum of these animals were removed, and part of these tissue were processed through paraffin section for Haematoxylin and Eosin (H & E).

Histological analysis

The cerebellum of the experimental rats were removed and preserved in labelled bottles containing 10% buffered formalin. These were allowed to stand for 72hours to achieve good tissues penetration and effective taxation. After this, they were placed in ascending grades of ethanol for dehydration. First they were treated with two changes of 70% ethanol each lasting for one hour followed by 95% ethanol and then absolute alcohol for the same duration. Following dehydration, tissues were cleared in three changes of xylene each lasting for fifteen minutes. Impregnation in molten paraffin wax at 58^{o}c was carried out overnight and the following morning the tissues were embedded in wax to form blocks. These tissue blocks were trimmed and sectioned at 5µ thickness using rotary microtome.

The section were floated in warm water (28oc) and then taken up on aluminized glass slides. They were air-dried and stained using the Haematoxylin and Eosin (Harris, 1990) staining method. Xylene was cleared in 95% alcohol for another minute. The section were washed well in running tap water for 15 minutes, differentiated in 1% alcohol for 5-10 seconds section turned blue. They were thereafter counter stained with 1% alcohol ascending grades of alcohol, Eosin for 1 minute. Followed by rapid dehydration through ascending grades of alcohol, cleared in xylene and mounted wit DPX mountain. Stained section were viewed under a light microscope and photomicrography.

Results

Statistical analysis

Statistical analysis was performed using one way ANOVA, followed by Bonferroni's multiple comparison test. Experimental data was presented as mean ± standard error of of mean (SEM). Valves of P0.05 were taken to be statistically significant.

GROUPS		Mean ± SEM	P-value
Control	Initial	125.0 ± 4.00	
	Final	165.0 ± 5.00	0.016*
Low dose	Initial	132.0 ± 2.00	
	Final	153.0 ± 3.00	0.149
High dose	Initial	138.5 ± 3.50	
	Final	152.5 ± 1.50	0.218

Table 1: Showing the Effect of Aqueous Extract of Theobroma cacao on Body Weight.

Result from the table above showed that there was a significant increase in body weight in the control group when comparing the initial weight to the final weight. For the low dose and high dose groups, the was insignificant increase in body when comparing initial to final weight (P>0.05)

		MEAN ±SEM	P-VALUE	F-VALUE
Relative Organ weight (g)	Control	1.25 ± 0.08		
	Low dose	1.41 ± 0.03	0.132	
	High dose	1.49 ± 0.07	0.046**	3.318

Table 2: Showing the effect of *theobroma cacao* on the relative weight of the cerebellum

Values are in Mean ± SEM (n=8). ** p<0.05 vs normal control is significant.

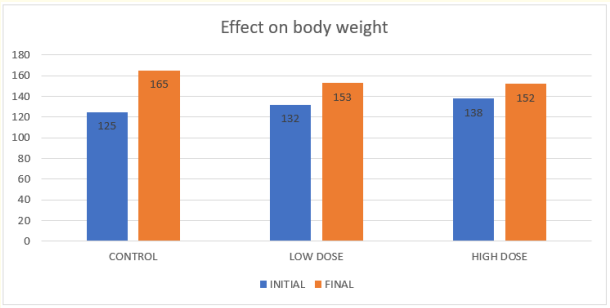

Figure 1: Bar Chart Showing the Effect of Aqueous Extract of Theobroma Cacao on Body Weight of Experimental Animal.

Result from the table above, the group administered high dose shows significant increase in the relative weight of the cerebellum while the low dose group, shows and insignificant increase in the relative organ weight.

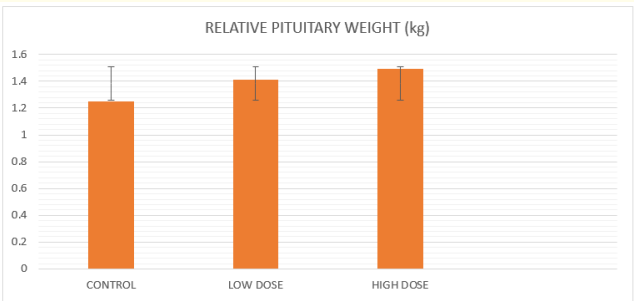

Figure 2: Bar chart showing the relative cerebellum weight of experimental animals.

Histological observations

Plate 1: Photomicrograph of cerebellum (Control) showing molecular layer (P) granular layer (F). (H&E x40).

Plate 2: Photomicrograph of cerebellum (Low dose) showing, scattered glial cells (P) and granular layer (FC) can be seen: No pathology seen. (H&E x40)

Plate 3: Photomicrograph of cerebellum (High dose) showing scattered glial cells (P) and many granular cells (FC) can be seen. no pathology seen (H&E x10).

Discussion

The use of plant as source of remedies for the treatment of many diseases dated back to prehistory and people of all continents have this old tradition. Despite the remarkable progress in synthetic organic chemistry of the twentieth century, over 25% of prescribed medicines in industrialized countries are derived directly or indirectly from plants [5].

From the result of the study carried out, it was observed when comparing the final weight to the initial body weight, that there was an insignificant increase in body weight in the test groups ($p>0.05$) this data suggest that the effects of *Theobroma cacao* on body weight and body composition could be mediated through changes in food intake rather than metabolic effects. This is in line with work done by David., *et al.* [6] who carried out a research on the effect of an herbal mixture on the body weight of wistar rats but is in contrast with work done by Muhammad and Ali [7], who studied the effect of herbal plant *Cydoniaoblonga* (a herbal plant) on adult wistar rats.

Organ weight is one of the most sensitive drug toxicity indicators, and its changes often precede morphological changes [8]. Organ background data is particularly important because they are not only used to determine whether treatment group animal organ weights are in the range of background data or not but also provide an important reference to provide to pathologists for gross anatomy and microscopic examinations [9].

From the result of the study above there was an insignificant increase in the relative weight of cerebellum. These insignificant increase, may be an indication that the extract did not cause inflammation at the cellular levels of the cerebellum this is in line with work done by Mohd. Nazrul Islam., *et al.* [10] who carried out a research on effects of an indigenous contraceptive herbal formulation on gonadotrophs of the pituitary gland of the rats.

Histological findings also supports, the claims reported in the relative weight above. Results revealed normal appearance of glial cells, granular cells, medullar of white matter and molecular cell layer all appearing normal with no sign of pathology which is in constrast with works done by Aquaisua., *et al.* [11] and Ekong [12] who studied the effect of some medicinal plants on the cerebellum.

Conclusion

From the results of the study above, it can be deduced that *Theobroma cacao* has no effect on the cerebellum of adult wistar rats at the dosages administered.

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