



# OVARIAN PROFILE OF WISTAR RATS TREATED WITH THEOBROMA CACAO EXTRACT

Kebe E. Obeten<sup>\*1</sup>, Victor A. Fischer<sup>2</sup>, Ugwuja O. Jennifer<sup>1</sup>, Akim Bonaventure<sup>1</sup>

<sup>1</sup>Department of Anatomy and Forensic Science, Cross River University of Technology, Okuku Nigeria

<sup>2</sup>Department of Anatomy, University of Calabar, Calabar

\*Correspondence to Kebe Obeten Email: [fredobeten@yahoo.com](mailto:fredobeten@yahoo.com), Tel. : +2348035505856

## ABSTRACT

The study was aimed at determining the effect of aqueous extract of *Theobroma cacao* on the histology of the ovary of female albino wistar rat. Twenty-four (24) adult wistar female rats weighting 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; the low dose group was administered 240mg/kg body weight of *Theobroma cacao* extract and the high dose group was administered 500mg/kg body weight of the test substance. Extract was given daily by oral gavage method for twenty-one (21) days. Twenty-four hours after the last administration, all animals in each group were sacrificed under chloroform anesthesia. The ovaries were harvested, weighed and fixed in 10% buffered formalin for histological studies. Results showed that following administration of extract of *Theobroma cacao* at these doses, an insignificant decrease in organ weight was observed. Histological observation showed few follicles as well as loss of the substance of granulosa cell this could possibly suggest decrease in production of sex steroids in the ovary.

**Keywords:** *Theobroma Cacao*, Ovary, Histology

## INTRODUCTION

Reproductive toxicity is increasingly becoming recognized as an important part of overall toxicology (Wang *et al.*, 2018). The commonly used conventional combined oral pills are usually associated with many untoward adverse effects, necessitating newer and indigenous drugs. The use of plants for medicinal and mythological purposes, and for solving problems related to ill health has been practiced in the African and other societies for many years (Mohammed *et al.*, 2014).

Plants have been used for medicine thousands of years and remain relevant as a natural source of active compound for treatments of human diseases especially cancer (Cragg and Newman, 2013). Many medicinal plants are used to treat various reproductive function ailments such as female infertility which is a public health concern in sub-saharan African countries (Lienou *et al.*, 2010). *Theobroma cacao* is a perennial

evergreen tree that originated from the neotropical rainforest, primarily in the Amazon basin and Guyana plateau (Bhatlacherjee and Kumar, 2007; Zhang *et al.*, 2011, Grassi and Ferri, 2012). It is classified in the genus *Theobroma* family Malvaceae sterculeacea and is considered a prehistorical tree already cultivated more than 3000 years ago by the olmecs and mayans (Dillinger *et al.*, 2000). Among their reported contributions for human health, some have been used traditionally for their Antioxidant (Jalil and Ismail, 2008), Antiinflammatory (Selmi *et al.*, 2008), Anticarcinogenic (Fotsis *et al.*, 1997; Maskarinec, 2009), Immunomodulatory, vasodilatory and analgesic (Wollgast and Anklem, 2010) and Antimicrobial (Summa *et al.*, 2008; Faphunda and Afolayan, 2012). The cultivation of *Theobroma cacao* is of economic importance to several countries like Ghana, Ivory Coast, Nigeria, Indonesia, Malaysia and Brazil (Azizah *et al.*, 2007; Hii *et al.*, 2009).

*Theobroma cacao* was used by ancient people as a medicinal plant for treating various disorders. Over 100 medicinal uses of *Theobroma cacao* have been documented in Europe and New Spain from 16<sup>th</sup> to the early 20<sup>th</sup> century; it has been used to treat Anaemia, mental fatigue, tuberculosis, fever, gout, kidney stones, low breast milk production and even poor sexual appetite (Dillinger *et al.*, 2000).

*Theobroma cacao* have been associated with several medicinal purposes (Dillinger *et al.*, 2000). In view of this, it became very necessary to carry out this research work with a view to ascertain the fertility potential of aqueous seed extract of *theobroma cacao* in the ovaries of female albino wistar rat.

## MATERIALS AND METHOD

### Extract preparation

*Theobroma Cacao* were harvested from a cocoa farm located in Orimkpong Emeh, Boki Local Government Area of Cross River State, Nigeria. The nuts 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 27°C 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 wire gauze cages 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 & 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 21 days. Group B (Low dose group) animals were treated with 240mgkg<sup>-1</sup> body weight of *Theobroma cacao* seed extract, while group C (High dose group) animals were treated with 500mgkg<sup>-1</sup> 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 ovaries of these animals were removed, and part of these tissue were processed through paraffin section for Haematoxylin and Eosin (H & E) staining.

### Histological analysis

The ovaries of the experimental rats were removed and preserved in labelled bottles containing 10% buffered formalin. These were allowed to stand for 72 hours to achieve good tissues penetration and effective fixation. 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°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 (28°C) and then taken up on aluminized glass slides. They were air-dried and stained using the Haematoxylin and Eosin (Harris, 1990) staining method for tissue blocks were section at 5µ with a rotary microtome. They were dew axed in xylene for 2 minutes per 2 changes. 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 photomicrograph of the stained tissue was taken.

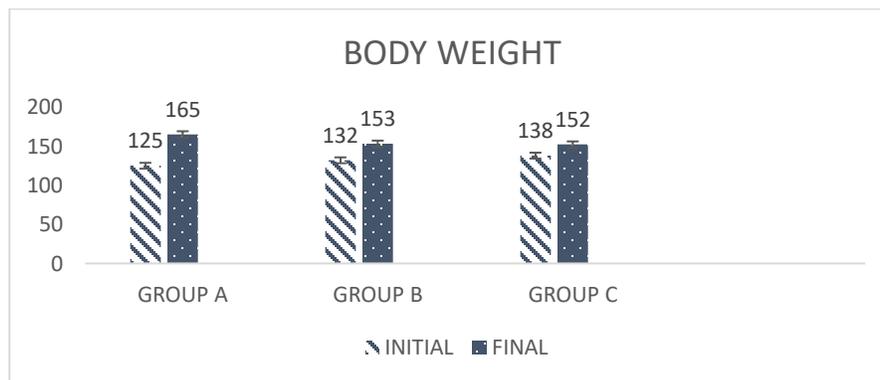
### Statistical Analysis

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.

## RESULTS

Results 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 (figure 1)

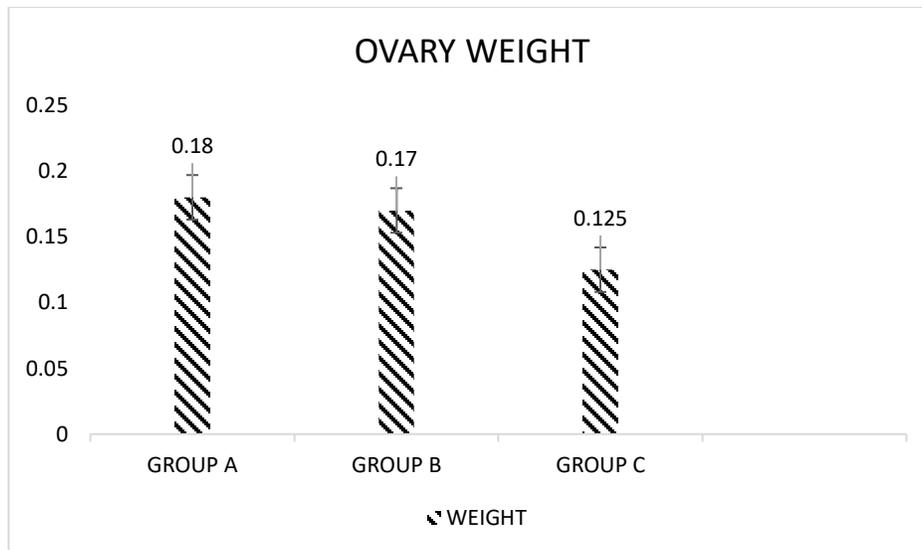
It was also observed that there was an insignificant decrease in the relative organ weight ( $P > 0.05$ ; figure 2)



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

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

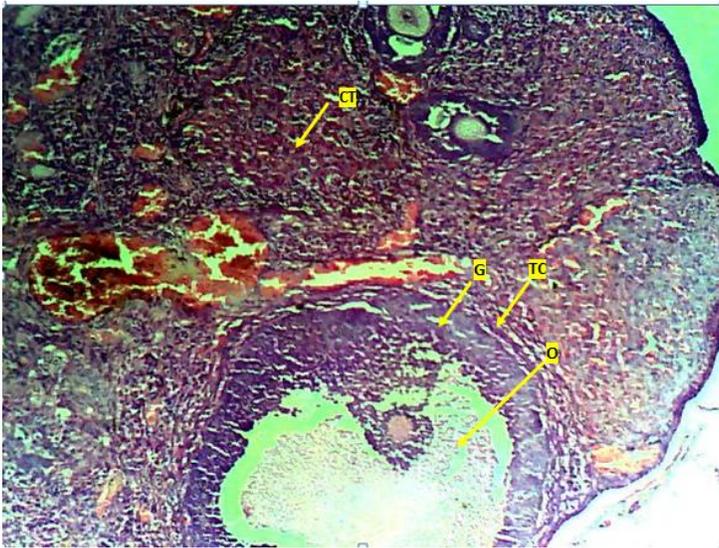
GROUPS		Mean ± SEM	P-value
CONTROL	Initial	125.0±4.00	0.016*
	Final	165.0±5.00	
LOW DOSE	Initial	132.0±2.00	0.149
	Final	153.0±3.00	
HIGH DOSE	Initial	138.5±3.50	0.218
	Final	152.5±1.50	



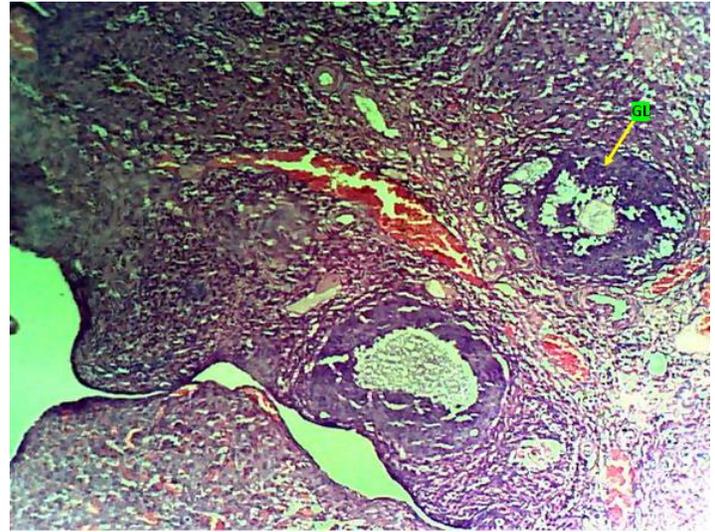
**Figure 2:** Bar Chart Showing the Relative Ovary Weight

**Table 2: Showing the effect of *Theobroma cacao* on relative ovary organ weight**

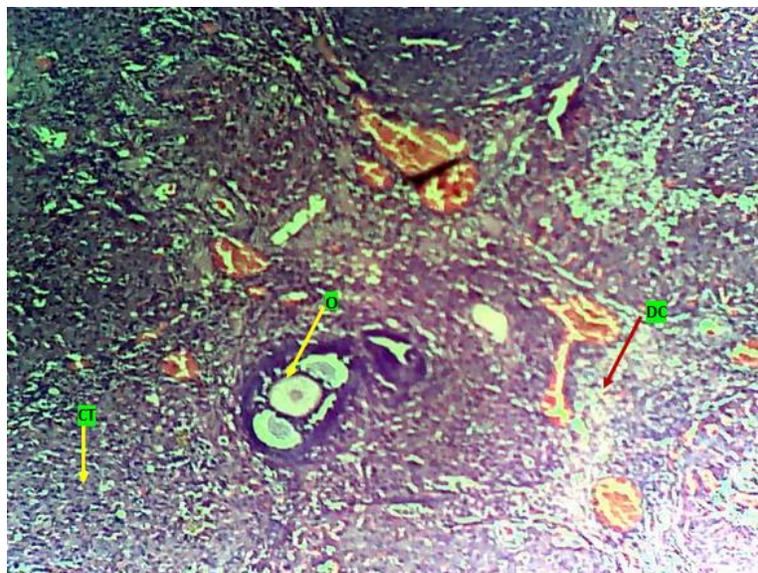
		MEAN	±SEM	P-VALUE	F-VALUE
Relative Organ weight (g)	Control	0.180	±0.100		
	Low dose	0.170	±0.300	0.750	
	High dose	0.125	±0.150	0.150	0.956



**Figure 3:** Photomicrograph of (control) ovary showing primary oocyte (O) surrounded by granulosa cells (G) and an outer theca cells (TC). The parenchyma is enclosed by connective tissue cells (CT) (H&E X40)



**Figure 4:** Photomicrograph of ovary of female wistar rat (low dose showing defragmented granulosa layer of an oocyte (GL) (H&E X40)



**Figure 5:** Photomicrograph of ovary of wistar rat (high dose showing oocytes (O) and defragmented connective tissue layer (DC). The connective tissue layer appears normal. (H&E X40)

## DISCUSSION

The use of plants for medicinal and mythological purposes, and for solving problems related to ill health has been practiced in the African and other societies for many years (Mohammed *et al.*, 2014). Some of the factors that help to increase the use of herbal medicines include its availability, cultural significance, history of known effectiveness, and most significantly, easy access compared to the modern pharmaceuticals (Thomford *et al.*, 2015).

Observation of body weight showed that there was a significant increase in body weight of animals in the control group; when comparing the initial weight to the final weight, but an insignificant increase in body weight was observed 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.*, (2007) who carried out a research on the effect of herbal mixture on the body weight of wistar rats but is in contrast with work done by Muhammad and Ali (2014), who studied the effect of herbal plant *Cydonia oblonga* on adult wistar rats.

Organ weight is one of the most sensitive drug toxicity indicators, and its changes often precede

morphological changes (Ying *et al.*, 2013). From the result of the study above, reduction in the observed weight of the ovary after treatment of the animals with *Theobroma cacao* seed extract may suggest the absence or reduced availability of ovarian hormone; this is in line with work done by Solomon *et al.*, (2010)

The result observed in the present study on the histology and follicular growth of the ovaries are also in line with other studies: Treatment of female rats with petroleum ether, benzene, chloroform and alcohol extracts of *Momordica charantia* seed extracts caused significant decrease in the number of developing follicles, Graafian follicles and corpora lutea and an increased number of atretic follicles in rats treated with these extracts as reported by Sharanabasappa *et al.*, (2002).

In conclusion, from the result of the study carried out above it is observed that the histological effect could be suggested that aqueous seed extract of *Theobroma cacao* showed various grade of degenerative changes, meanwhile prolong intake at high concentration has deleterious and adverse effect on ovaries of female wistar rats and this may impose danger in female reproductive profile.

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