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# Investigating the effect of *Centella Asiatica* on placenta and pregnancy outcome in rat experimental model

Akporobo Ejeguo\*, Christopher Lucky Sakpa

## ABSTRACT

Plant research and use for purposes other than food has been available for thousands of years. Such use includes the treatment of diseases and illnesses as well as for dermatological purposes. Traditionally, several specific ailments have been cured with different parts of medicinal plants. *Centella Asiatica* is widely known as 'Gotu kola' in Nigeria and is growing in the riverine areas of Bayelsa, Rivers, and Delta State. The leaves of *C. Asiatica* have been reported to possess several medicinal properties. This has resulted in its wide acceptance among several populations. This study investigated the effect of ethanolic leaf extract of *C. Asiatica* on pregnancy outcome and placenta. Forty-eight female Wistar rats with regular estrous were employed in this study. They were mated and confirmed pregnant before administration of the extract. They were divided into two groups that received treatment from GD7-13 and GD14-20. The subgroups received 250mg, 500mg, and 1000mg/kg body weight of the extract respectively while the control received 1ml of distilled water. 50% of the animals were sacrificed at GD20 while the others were left to litter. After sacrifice, the uterine horns containing the fetuses were harvested and examined for gross morphological variations. The fetal and placental weights were recorded for statistical analysis. The excised placenta was fixed in formal saline and taken through histological processing. Data obtained was analyzed using Statistical Package for Social Sciences (SPSS) and compared with ANOVA. There were no gross morphological variations in pups of treated dams, however, there was a dose-dependent decrease in the fetal weights indicative of intrauterine growth restriction (IUGR). The histology of the placenta junctional zone showed a dose-dependent reduction in the amount of glycogen cell islands while the labyrinth zone showed congestion of the maternal sinusoids.

**Keywords:** Pregnancy outcome, Placenta, *Centella Asiatica*, IUGR

## 1. INTRODUCTION

Pregnancy outcome is defined as the final result of a fertilization event. There are various outcomes of pregnancy and they include live birth, stillbirth, spontaneous abortion, induced abortion, and low birth weight (growth restriction). Of the live birth, there may also be cases of congenital abnormalities or birth defects. Most of these are adverse pregnancy outcomes and it has been reported that their incidence is greater in developing countries (Rosenfield and Maine, 1985). The reproductive system has several conditions that affect its optimum function resulting in infertility. *Centella Asiatica*, called *Centella Asiatica* (L.) Urb. or Gotu kola, is an herb used in traditional Chinese medicine in China and Southeast Asia to treat different diseases. The plant has a creeping stem, rooting at the nodes, producing tufts of leaves and white or pink flowers Chandrika and Prasad-Kumarab, (2015); it is used as a herbal drink, and consumed in Asian countries as a form of vegetable in local cuisines (Tassanawat et al., 2013).

In Ayurvedic and Chinese traditional medicine, *C. Asiatica* (CA) has been widely utilized for centuries to treat various dermatological conditions, such as scleroderma, bacterial infections, psoriasis, leprosy, ulcers, and skin inflammation caused by burns and wounds (Han et al., 2012; Jia and Lu, 2008; Kwon et al., 2010; Thomas et al., 2010; Kai et al., 2008). Furthermore, *C. Asiatica* is reported to possess neurological actions, including memory-enhancing, neuroprotective, anxiolytic effects, and antidepressant (Hengjumrut et al., 2018; Khemawoot et al., 2018; Han et al., 2012; Jia and Lu, 2008). In Nigeria, it is found around the coastal areas such as Bonny Island. Analytical studies have revealed that *C. Asiatica* contains triterpenoids, amino acids, and essential oils. Its key bioactive components include triterpene glycosides (saponins) such as Asiatic acid, and Madecassic acid (Wu et al., 2012; Plengmuankhae and Tantitadapitak, 2015).

Animal experiments have found that *C. Asiatica* extract has anti-spermogenic and anti-fertility effects on the reproductive system of male rats (Yunianto et al., 2010). The effect of a potential teratogen on embryogenesis can be gene mutation, chromosome breakage or non-disjunction, inhibition of substrates, depletion of energy sources, inhibition of enzymes, or changes in the intracellular milieu (Edwards, 1986). The placenta is a specialized organ critical to supporting pregnancy. It supplies the fetus with nutrients and oxygen while eliminating waste products and carbon dioxide. It also establishes a barrier between maternal and fetal circulation (the placental barrier). Furthermore, the placenta has an endocrine function as it secretes hormones human chorionic gonadotropin, that affect pregnancy, metabolism, fetal growth, and parturition (Bouw et al., 1976). Thus, the objective of this study was to investigate the effect of *C. Asiatica* on the placenta and pregnancy outcome in adult female Wistar rats.

## 2. MATERIALS AND METHODS

### Collection of plant materials and extraction

The leaves of *C. Asiatica* were obtained from a farm in Bonny Island of Rivers State, Nigeria. It was verified at the Department of Plant Biology and Biotechnology of the University of Benin. The *Centella Asiatica* was dried in the oven at 100 degrees Celsius for about 4 hours. The dried *Centella Asiatica* was then pulverized to powder form. The powdered *Centella Asiatica* (1.00kg) was extracted thrice in ethanol (10 L) at room temperature on a shaker for 48 hours. Filtration of the extract was done with a Buchner funnel and Whatman No.1 filter paper. The extract filtrate was rapidly frozen at -40°C and dried for 48 hours using a freeze dryer. The dried extract was then reconstituted with distilled water to achieve the desired concentrations for the study.

### Experimental animals

Forty-eight female Wistar rats were used for this study and kept in polypropylene cages at room temperature. They were purchased from the Department of Anatomy, University of Benin. The animals were given access to feed and clean water and allowed to acclimatize for two weeks before the commencement of the experiment. They were maintained under standard conditions for laboratory animals (National Institute of Health Guide for the Use and Care of Laboratory Animals).

### Mating of Animals

The animals' estrous cycle was monitored and animals were mated on the night of their estrous phase. The animals were paired overnight at the estrous cycle with sexually active males in the ratio of 2:1. On the morning after mating, the presence of a plug and/or

sperm in the vaginal smear is considered as successful mating and termed GD0 (gestational day 0). Mated animals were divided into two groups that received treatment from GD7-13

### Experimental design

Group 1- control received 1ml of distilled water

Group 2- 250mg/kg body weight of *Centella Asiatica*

Group 3- 500mg/kg body weight of *Centella Asiatica*

Group 4- 1000mg/kg body weight of the extract respectively

50% of the animals were sacrificed at GD20 while the others were left to litter.

### Sacrifice

On the day of the sacrifice, the uterine horns were exposed to ensure that the following data was recorded: number of fetuses, fetal weight, fetal crown-rump length, placenta weight, placenta major diameter, and placenta minor diameter. The placenta was also harvested, placed in plain bottles containing 10% buffered formalin, and taken for histological processing. The obtained sections were demonstrated using H & E stain and photomicrographs of tissue sections were taken with a compound light microscope and digital camera.

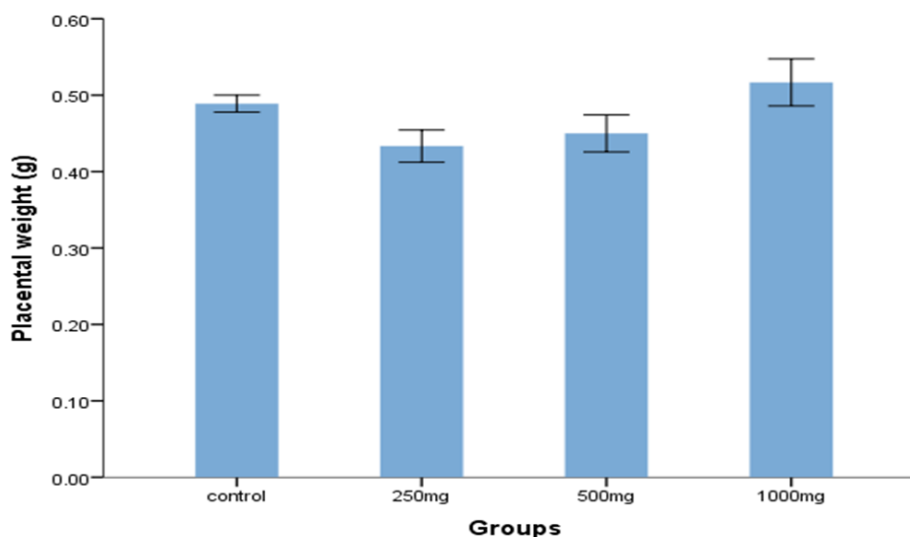
### Data Analysis

The analysis was conducted using IBM SPSS Statistics software, version 22 (IBM Corp., Armonk, NY, USA). A one-way analysis of variance (ANOVA) with post hoc Tukey tests was performed for mean comparisons, and graphs were utilized to present the results. Statistical significance was set at  $p < 0.05$ .

## 3. RESULTS

### Placenta weight

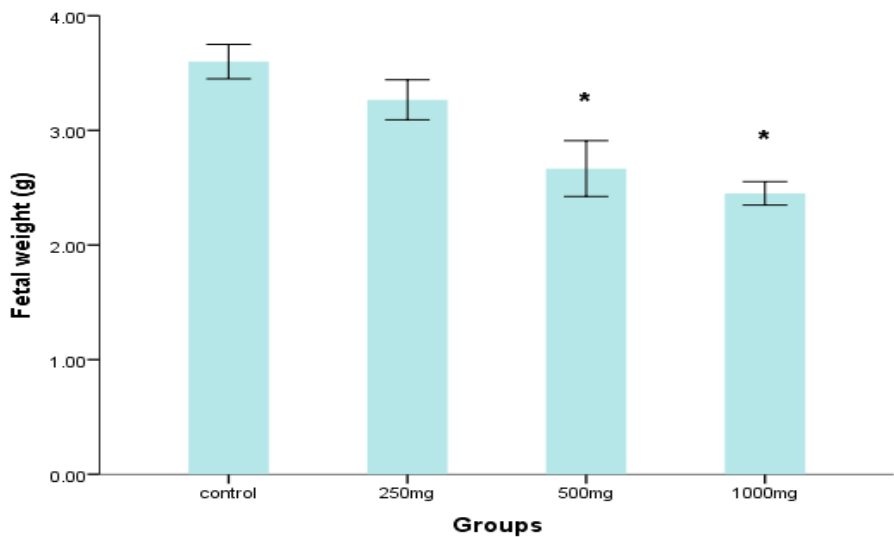
No statistically significant difference in the placenta weights in all treated groups compared to control;  $p=0.412$   $p=0.564$   $p=0.680$  for 250mg, 500mg, and 1000mg respectively (Graph I).



Graph I Placenta weight

Fetal weight

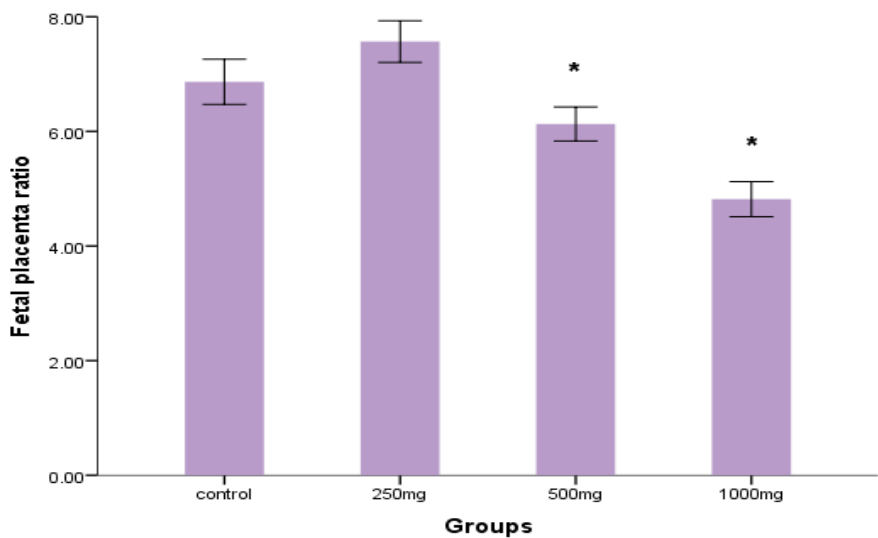
A statistically significant decrease in the fetal weight of group 500mg and 1000mg ( $p=0.000$  and  $p=.008$  respectively). There was no significance for group 250mg ( $p=0.709$ ), (Graph II).



Graph II Fetal weight (\* indicates  $p<0.05$ )

Fetal-placenta-ratio

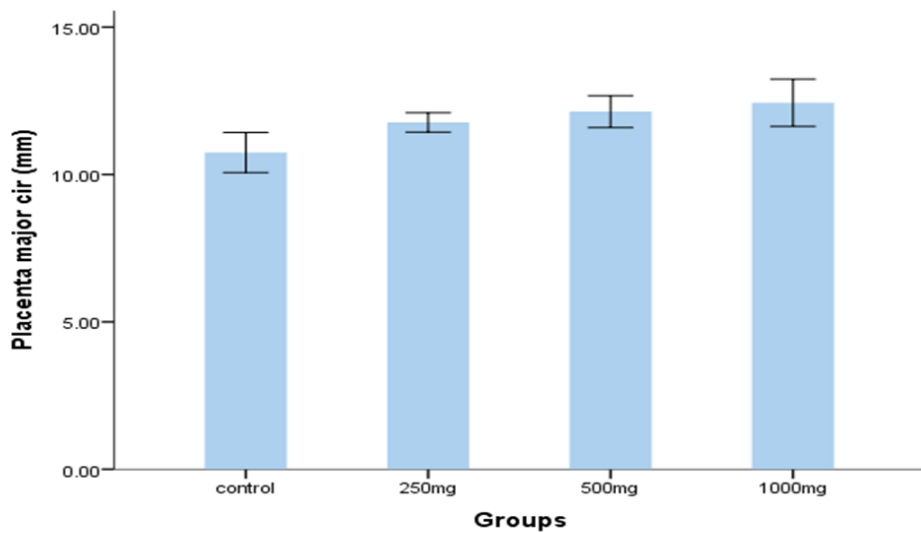
A statistically significant decrease in the fetal/placenta ratio of group 1000mg and 500mg  $p=0.005$  and  $p=0.005$  respectively. There was no significance for group 250mg ( $p=0.353$ ), (Graph III).



Graph III Fetal-placenta-ratio (\* indicates  $p<0.05$ )

Placenta major diameter

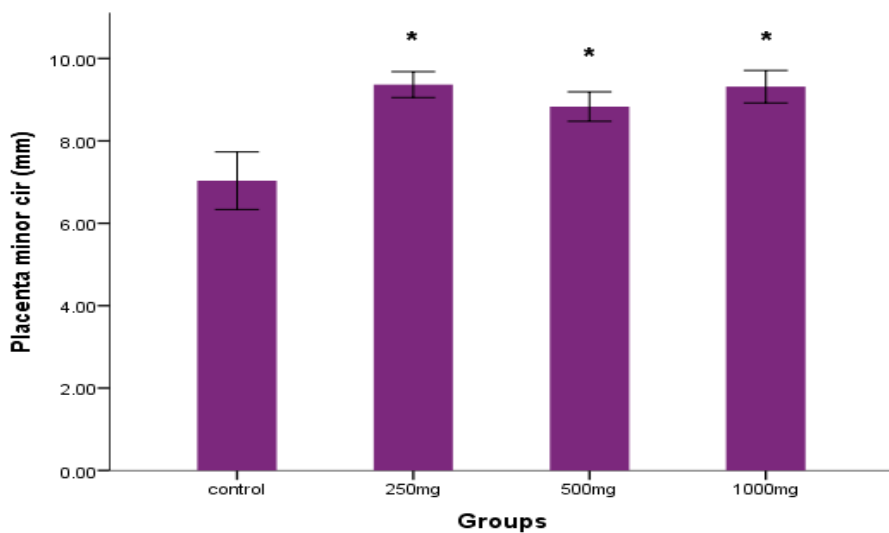
No statistically significant difference in the placenta major diameter in all treated groups compared to control;  $p=0.259$ ,  $p=0.129$ , and  $p=0.068$  for 250mg, 500mg, and 1000mg respectively, (Graph IV).



Graph IV Placenta major diameter

Placenta minor diameter

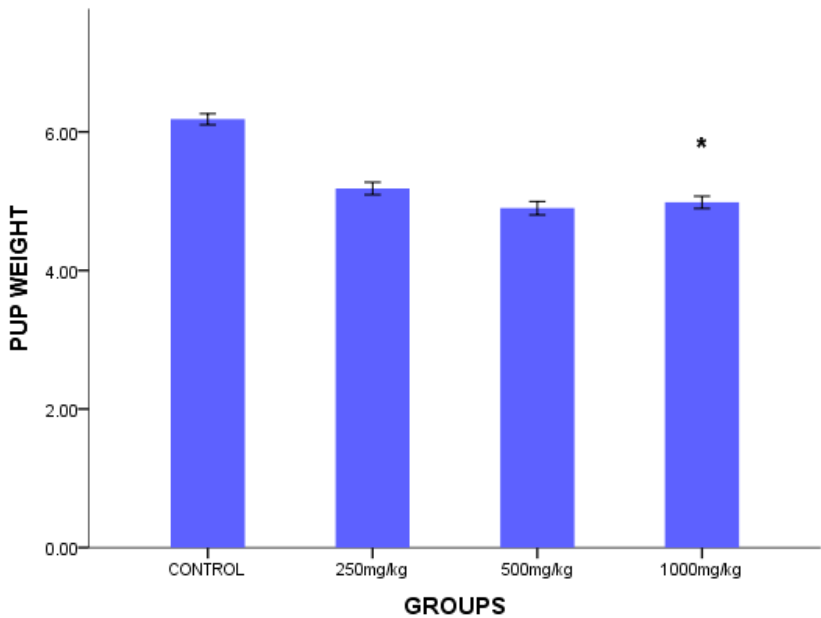
A statistically significant increase in all treated groups compared to control;  $p=0.005$ ,  $p=0.25$ , and  $p=0.006$  for 250mg, 500mg, and 1000mg respectively (Graph V).



Graph V Placenta minor diameter (\* indicates  $p<0.05$ )

Birthweight

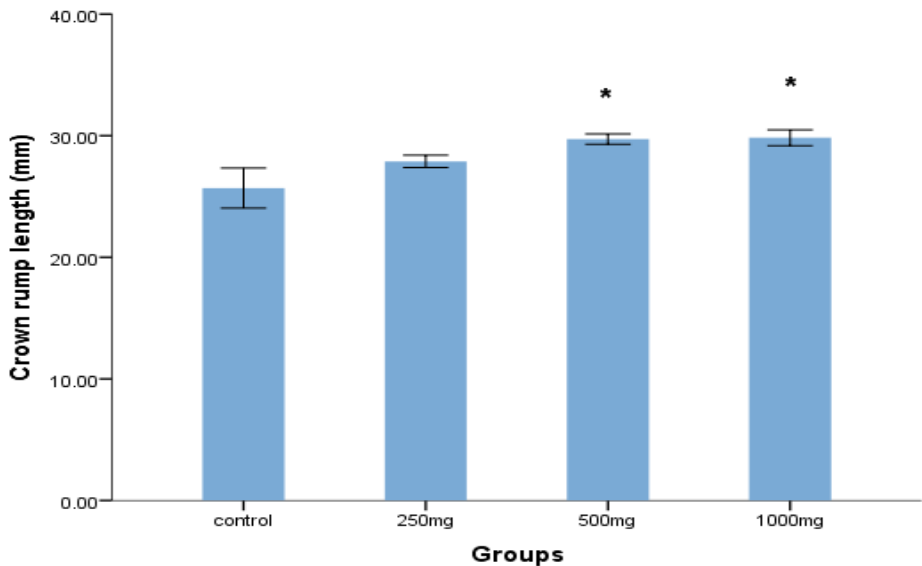
No statistical difference in the bodyweight of litters in the fetal phase compared to control. P= 0.400 p=0.052 p=0.431 for 250mg, 500mg, and 1000mg respectively, (Graph VI).



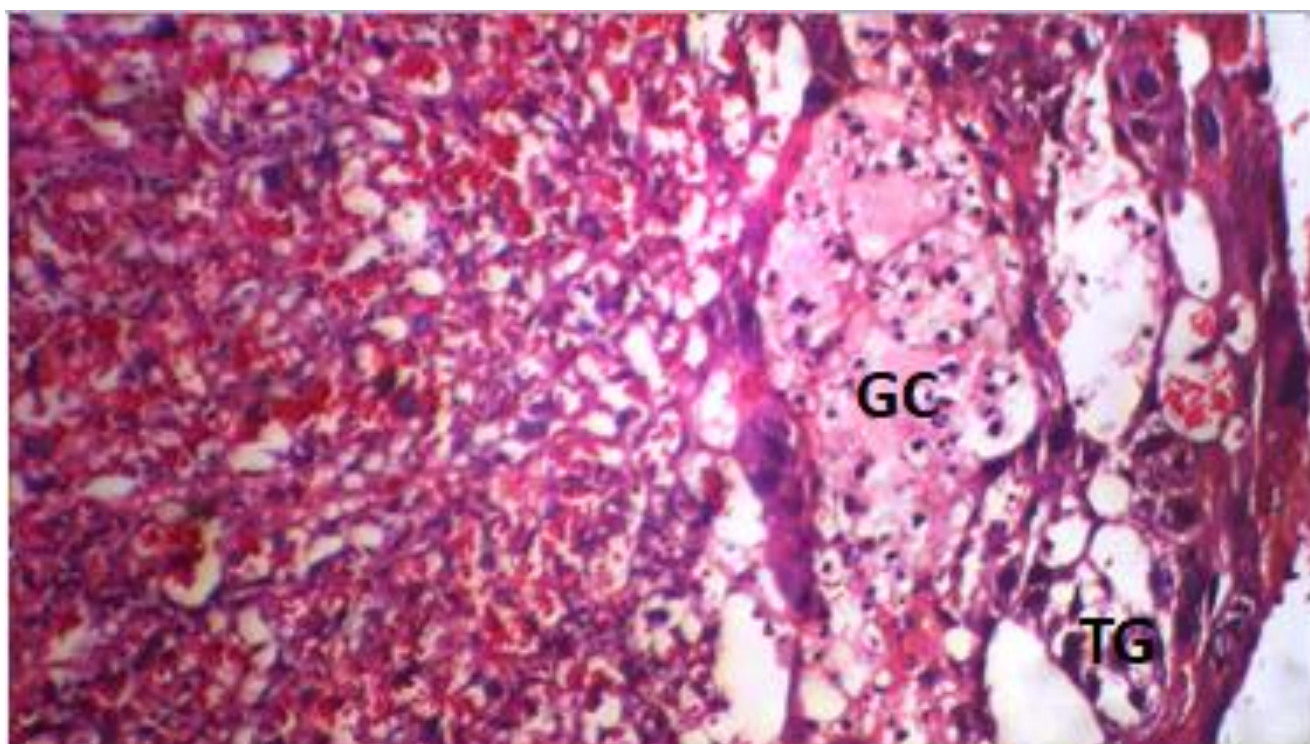
Graph VI Birthweight (\* indicates p<0.05)

Crown-rump length

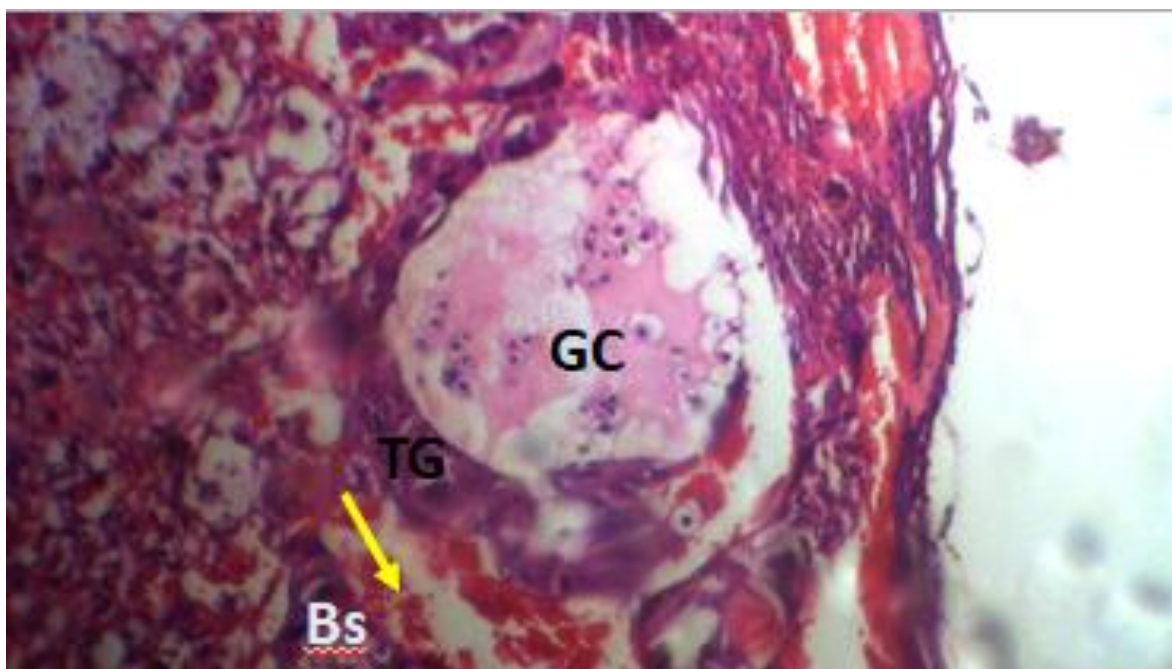
A statistically significant increase in the CRL in the 500mg and 1000mg (p=0.021 and p=0.018 respectively). However, there was no statistical difference in the 250mg (p=0.191), (Graph VII).



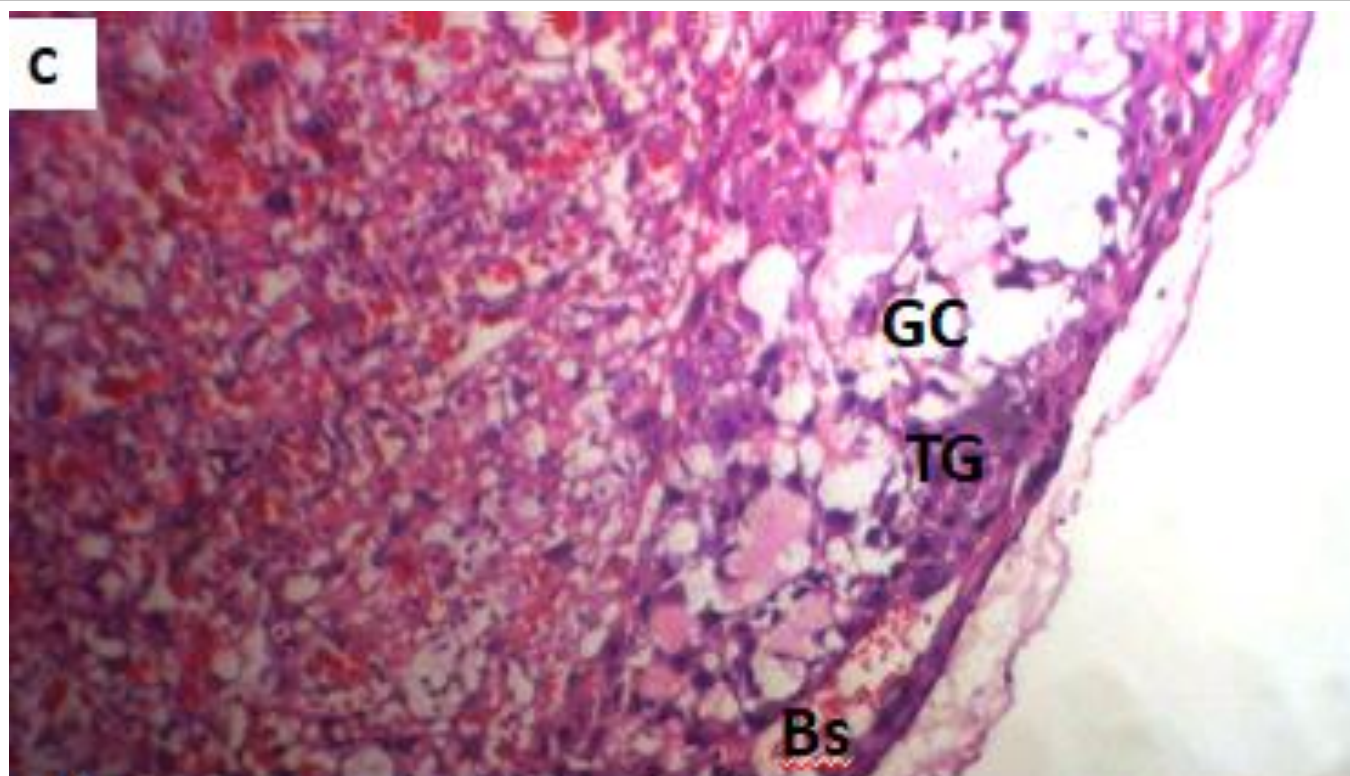
Graph VII Crown-rump length (\* indicates p<0.05)



**Plate I** Placenta junctional zone 100x: Group A-control, presence of abundant glycogen accumulation on the maternal side  
GC- glycogen cell island; TG-trophoblastic giant cells

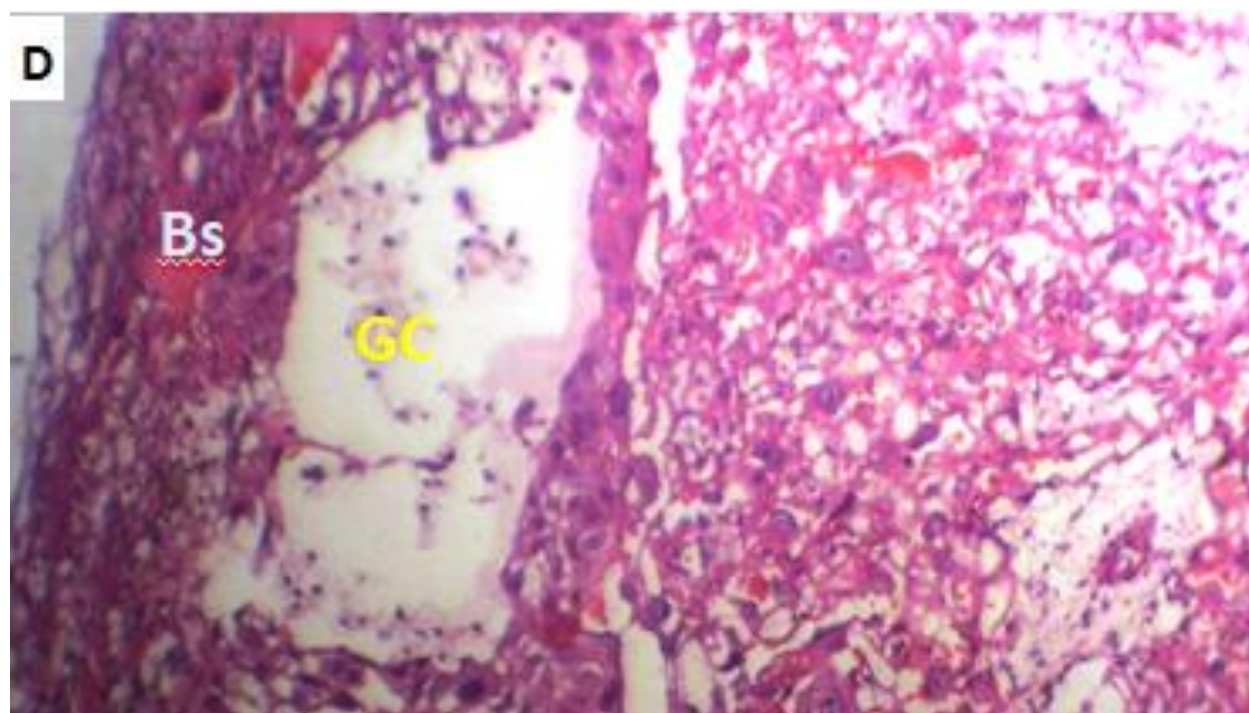


**Plate II** Placenta junctional zone 100x: Group B-250mg/kg, shows degeneration of glycogen cells evident as the presence of vacuoles in GC and interstitial congestion in Bs  
GC- glycogen cell island; TG-trophoblastic giant cells; Bs-blood sinusoids

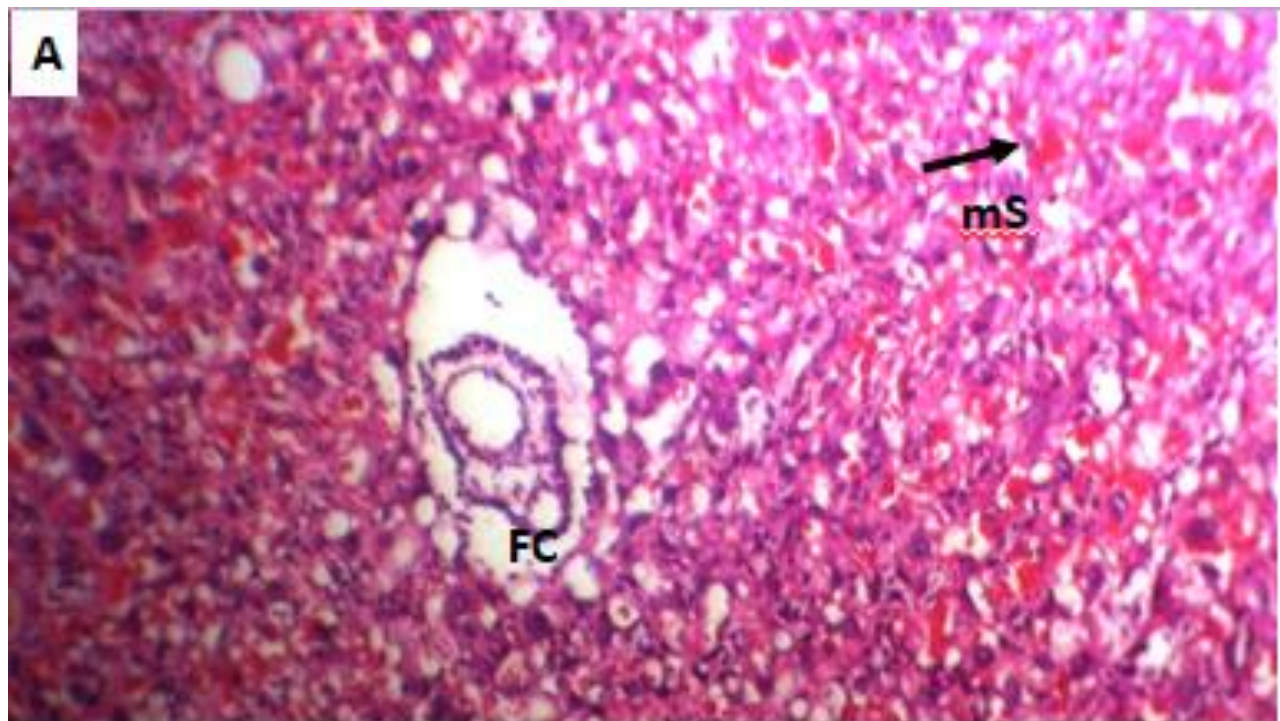


**Plate III** Placenta junctional zone 100x: Group C-500mg/kg, shows increased degeneration of glycogen cells evident as the presence of larger vacuoles in GC and interstitial congestion in Bs

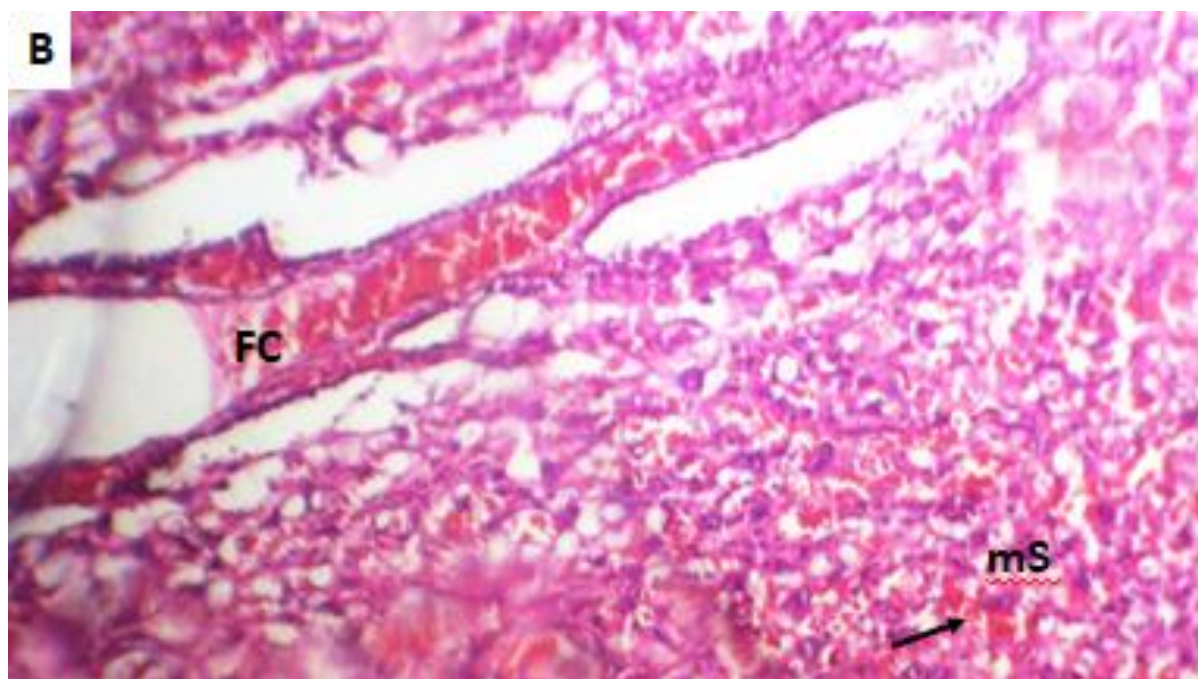
GC- glycogen cell island; TG-trophoblastic giant cells; Bs-blood sinusoids



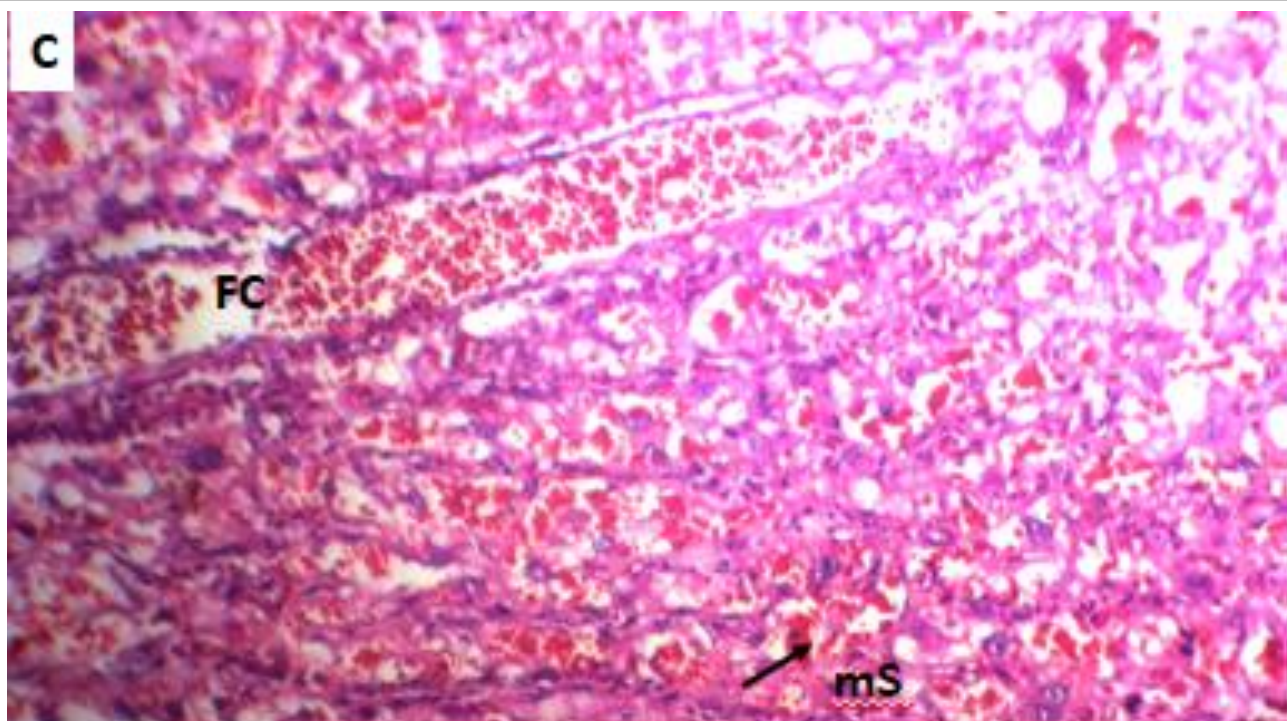
**Plate IV** Placenta junctional zone 100x: Group D-1000mg/kg, shows intense degeneration of glycogen cells evident as the presence of confluence of vacuoles in GC and interstitial congestion GC- glycogen cell island; TG-trophoblastic giant cells; Bs-blood sinusoids



**Plate V** Labyrinth zone of placenta 100x: Group A-Control shows normal fetal capillary and maternal sinusoids  
FC-fetal capillary; mS-maternal sinusoids

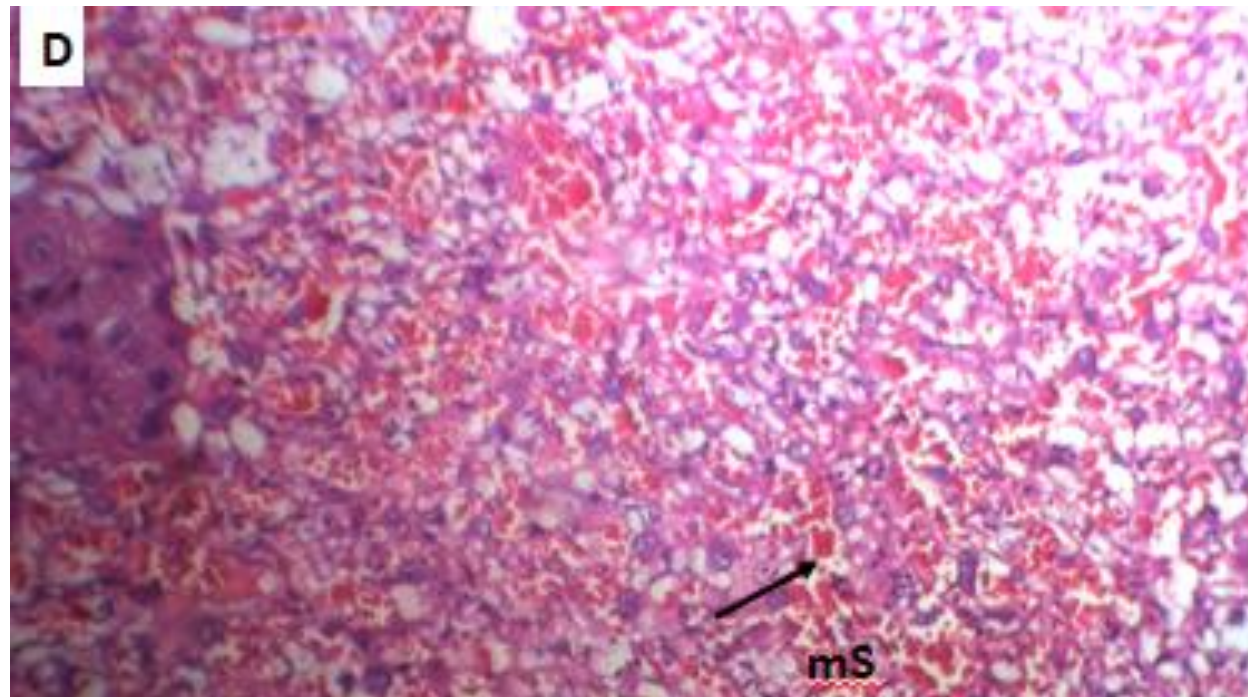


**Plate VI** Labyrinth zone of placenta 100x: Group B-250mg/kg shows dilated and congested fetal capillary and mild interstitial congestion  
FC-fetal capillary; mS-maternal sinusoids



**Plate VII** Labyrinth zone of placenta 100x: Group C-500mg/kg shows increased dilation and congestion of fetal capillary and moderate sinusoidal congestion

FC-fetal capillary; mS-maternal sinusoids



**Plate VIII** Labyrinth zone of placenta 100x: Group D-1000mg/kg shows intense dilation of fetal capillary and sinusoidal congestion

## 4. DISCUSSION

The study showed that *C. Asiatica* had no significant effect on the weight of the placenta whereas there was a notable decrease in the fetal and birth weights. The placenta is an essential organ for the development and growth of the fetus. It is considered a semi-permeable barrier that regulates the transfer of nutrients, wastes, gases, and endogenous and exogenous molecules between maternal and fetal circulations. However, it has been established that the placenta is highly permeable to substances with diverse molecular structures that can readily cross from the maternal blood to reach the fetus (Prouillac and Lecoœur, 2010). Exogenous agents, such as medications, toxins, herbs, and environmental factors, can impact placental function and fetal weight. These factors can alter the normal development and functioning of the placenta, leading to reduced nutrient and oxygen supply to the fetus (Bloise et al., 2014).

Disruption of this cascade can lead to abnormal development of the placental vasculature or the trophoblast. Timing of a developmental 'insult' will be critical in consequent placental function and hence conditioning of the fetus. Factors that disrupt placental development, such as hypoxia and abnormal maternal nutrient levels, can prompt the placenta to adapt. These adaptations may involve changes in transporter expression and activity to support fetal growth or epigenetic modifications regulating placental gene expression (Myatt, 2006). As a result, the fetus may experience restricted growth and lower birth weight. Additionally, some exogenous agents can directly affect the growth and development of the fetus by crossing the placental barrier.

For example, certain medications or drugs can have teratogenic effects, causing developmental abnormalities and impairing fetal growth (Myatt, 2006). The fetus is at the end of a supply line that ensures delivery of nutrients from the maternal/uterine circulation to the fetus via the placenta. Fetal-placental exchange of nutrients is subject to the placental architecture, size, developmental, and pathological processes both qualitatively and quantitatively. Placental and fetal weights are thought to be closely linked, with studies showing a positive correlation between placental weight and birth weight in infants of normal and large gestational age (Cetin, 2011). Hormones from the fetal somatotrophic axis, especially insulin-like growth factor (IGF)-1, play a significant role in regulating fetal growth (Bauer et al., 1998).

However, the specific effects of exogenous agents on placental function and fetal weight can vary depending on the agent and the timing of exposure. For example, exposure to certain medications during early pregnancy may have a greater impact on fetal development and weight compared to exposure later in pregnancy. Additionally, decreased progesterone following the administration of *C. Asiatica* levels can disrupt the normal hormonal balance necessary for maintaining a healthy pregnancy (Filant and Spencer, 2014). This disruption may lead to complications such as insufficient uteroplacental vascular development, abnormal trophoblast invasion, and irregular placental formation. These complications can hinder fetal growth and development by impairing the vascular remodeling of uteroplacental tissues, resulting in reduced blood flow and oxygen delivery to the fetus.

As a result, the fetus may experience inadequate nutrient and oxygen supply, which may lead to restricted growth and lower fetal weight. Osifo and Ezeuko, (2024) reported that the fetal-to-placental weight ratio is a metric used to assess placental efficiency, which is crucial for understanding fetal growth and development. Changes in fetal-placenta ratio can indicate disruptions in placental function, potentially leading to nutrient insufficiency for the developing fetus. Our findings coincide with a study by Azza et al., (2018), who observed a highly significant decrease in placental weight of viable fetuses in both treated groups of the drug aspartame. This result was also in agreement with those obtained by (Portela et al., 2007; Leme and Azoubel, 2006; Martins and Azoubel, 2007).

Azza et al., (2018) also reported that doses of 14mg/kg, and 40mg/kg of aspartame daily showed a significant decrease in fetal weight when both groups of aspartame-treated rats were compared with the control group. This reduction could indicate that the fetuses could not get their requirements of substrates, including glucose, which might be due to the possible diminution of substrates in the blood of maternal rats that utilized the sweetener, and in this case, *Centella Asiatica*. Again, this was similar to the results of other researchers including (Leme and Azoubel, 2006; Martins and Azoubel, 2007; Portela et al., 2007). Our findings also reported an increased placenta minor diameter compared to control for all administered groups of *C. asiatica*. Abnormal placenta size and shape indicate abnormal neonatal birth weight via gaps between fetal nutritional requirements and placental supply (Ravikumar et al., 2018).

Specifically, lower placental weight, smaller placental volume and area, shorter major and minor axes, and UC length at birth are risk factors for complicated fetal growth restriction. Ouyang et al., (2013) established that the percentile of placental weight at delivery may be utilized to identify the etiology of fetal intrauterine growth limitation, supporting the association between neonatal weight and placental weight at birth. The surface area is a crucial parameter for fetal development, given its role in oxygen diffusion and nutrient

transfer. According to research by Ravikumar et al., (2018) an eccentric placenta with an apparent non-long minor axis has a reduced placental size and is associated with intrauterine growth limitation in small gestational-age infants.

According to research by Alwasel et al., (2012) who examined the connection between placental form and fetal development, he reported that placental width (minor axis) positively correlated with the placental area, perimeter, and body size at delivery. The size of the placental surface at birth, but not its length, is linked to the development of four prevalent illnesses in later life: Hypertension Barker et al., (2010a); lung cancer Barker et al., (2010b), chronic heart failure Barker et al., (2010c), and coronary heart disease (Eriksson et al., 2011). Roseboom et al., (2011) reported that in Holland, in males in utero during the famine hypertension was predicted by a large placenta breadth (minor diameter). The histology of the placenta from our results showed a reduction in glycogen cell islands in the treated groups.

One function of the placenta is to facilitate glucose transport, it metabolizes it for its use and stores it up as glycogen. There is a hypothesis from animal studies that placenta glycogen stores serve as a source of glucose to sustain fetal growth during late gestation. Credence to this hypothesis is derived from the observation that glycogen is the main energy storage in animals, and glycogen storage diminishes towards the end of pregnancy when accelerated fetal growth exists. The mouse placenta stores glycogen in a specific trophoblast cell subtype known as the glycogen trophoblast (GlyT), some of which have frequent contact with the maternal decidua (Coan et al., 2006). GlyT appears in the junctional zone of a mature placenta in clusters (Adamson et al., 2002).

Understanding placental glycogen function using genetic mouse models, there is evidence linking altered placental glycogen storage to human pregnancy complications. This reaffirms the important role of glycogen in achieving a successful pregnancy outcome (Akison et al., 2017). In mice, fetal growth restriction (FGR) is evident in most mouse models exhibiting knockout or reduced expression of genes associated with GlyT, which authenticates the hypothesis that placental glycogen contributes to fetal growth support (Plates I, II, III, & IV). The labyrinth zone contains trophoblastic septa, which consist of fetal capillaries, trilaminar trophoblastic epithelium, and maternal sinusoids (Plates V, VI, VII & VIII). These maternal sinusoids which carry the mother's blood are between the trophoblastic septa.

The cytotrophoblast refers to the trophoblast epithelium that interfaces with the maternal sinusoids. The fetal capillaries are fenestrated, allowing for high permeability. In this region, maternal and fetal blood come into proximity, facilitating most maternal-fetal substance exchange. As pregnancy advances, the labyrinth zone plays a crucial role in this exchange (Wooding and Burton, 2008). The continuity of the syncytiotrophoblast layer provides a placental barrier (Georgiades et al., 2002). Intrauterine growth retardation (IUGR) as seen from the decreased fetal weight of this study is highly correlated with injury to the labyrinth zone. Due to its higher blood flow, more cellular proliferative activity, and longer proliferation time than other placental regions, the labyrinth zone is more susceptible to the target site in placental toxicity (Furukawa et al., 2015).

Labyrinth zone hypertrophy is linked to a rise in trophoblast mitosis and maternal sinusoidal dilatation antiestrogenic agents like *C. Asiatica*. During the final phase of pregnancy, growth and functional maturation of fetal organs were established during the embryonic stage. The most common anomalies associated with fetal-stage exposure to teratogens are growth restriction and behavioral changes (Gilbert-Barnes, 2010). A decrease in birth weights at 500mg and 1000mg and a significant increase in crown-rump length (CRL) was observed in both groups. This reduction in the birth weight indicates that the fetuses could not obtain their requirements of substrates, including glucose, which might be due to the possible diminution of substrates in the blood of maternal rats that utilized *Centella Asiatica*. Again, this was similar to the results of other researchers such as Leme and Azoubel, (2006), Martins and Azoubel, (2007) and Portela et al., (2007) following administration of the drug aspartame.

## 5. CONCLUSION

In conclusion, *Centella Asiatica* caused various developmental derangements such as reduced fetal weight and fetoplacental ratio. It led to alteration of the histo-architecture of the placenta especially on the reduced glycogen cell islands, and sinusoidal congestion. All these point to the fact that the fetus did not receive adequate nutrients necessary for its growth and development. The extract of *Centella Asiatica* is, therefore, generally unfit for consumption during pregnancy.

**Author's Contribution**

All authors were actively involved from the commencement of the work till its completion. They all participated in the design, execution, interpretation, writing, and editing of the manuscript.

**Acknowledgment**

We sincerely appreciate the Department of Anatomy for making their animal house and laboratories available.

**Ethical approval & declaration**

In this article, as per the animal regulations followed in Department of Anatomy, University of Benin, Benin City, Nigeria, the authors investigating the effect of *Centella Asiatica* on placenta and pregnancy outcome in rat experimental model. The experimental animals were maintained under standard conditions for laboratory animals (National Institute of Health Guide for the Use and Care of Laboratory Animals). The Animal ethical guidelines are followed in the study for species observation, & experimentation. Also, the ethical guidelines for plants & plant materials are followed in the study for plant collection, identification & experimentation.

**Informed consent**

Not applicable.

**Conflicts of interests**

The authors declare that there are no conflicts of interests.

**Funding**

The study has not received any external funding.

**Data and materials availability**

All data associated with this study are present in the paper.

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