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Research Article

Evaluation of Leaf Extracts of *Costus afer* on Foetal Morphology and Cerebral Cortex Using Rat Model

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Abstract

Background and Objectives: The use of medicinal plants and different herbs in the treatment of different ailments by the populace including pregnant women may have some teratogenic effects on the fetuses. *Costus afer* is a medicinal plant used in tropical Africa in treating inflammation, rheumatism, arthritis, hepatic disorders, miscarriages, epileptic attack and hemorrhoids. This study is aimed at evaluating *Costus afer* on fetal morphology and cerebral cortex of albino rats. **Materials and Methods:** Twenty-four female rats weighing between 150 and 180 g divided into 4 groups; Group A (Control), Group B (Low dose), C (Medium dose) and D (High dose) were used. The female rats were mated with mature male rats for pregnancy. Groups B, C and D animals received orally 250, 500 and 1000 mg kg⁻¹ b.wt., of ethanolic leaf extracts of *Costus afer*, respectively on days 7-12 of gestation. On day 19 of gestation, the rats were sacrificed and fetuses examined. **Results:** Results showed no observable malformation on the external structures of the fetuses. Reduction in the weight, crown-rump length and tail length of the fetuses in treated group B, C and D, when compared to the control group was not significant ($p > 0.05$). Results from the histological examination by using Haematoxylin and Eosin staining technique showed no evidence of alteration of the cerebral cortex of the treated groups when compared to the control group. **Conclusion:** Ethanolic leaf extract of *Costus afer* appears to be safe for use during pregnancy with probably no toxic effects on the morphology and histology of the cerebral cortex of fetuses of albino Wistar rat.

Key words: *Costus afer*, crown-rump, tail length, morphology, cerebral cortex

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Majority of people living in developing countries depend on traditional and medicinal plants, which are cheap, abundant and at the same time culturally acceptable¹. The leaves and stems of these plants are used in the preparation of traditional medicine used in the treatment of several ailments². Some medicines used during pregnancy might have harmful impact across the placenta barrier and cause congenital malformations which place a considerable burden on the affected child³. The effects of herbs on the embryo and fetus are not known in many cases. It is possible that herbal chemicals may be transported through the placenta to cause toxic effects on the sensitive growing fetuses⁴. Approximately 1 in 250 newborn infants have structural defects caused by an environmental exposure and presumably, a larger number of children have growth retardation or functional abnormalities resulting from non-genetic causes, in other words, from the effects of teratogens. Most of the structural defects occur during organogenesis due to exposure to teratogens. This is the critical period when organ systems are been established⁵.

Research has shown that some plants and drugs are teratogenic on the fetuses and the evaluations of teratogenic potentials of medicinal plants are important for the safeguard of the fetus^{6,7}. *Momordica foetida* schum (Cucurbitaceae) is a medicinal plant that has been used for the treatment of severe headache, earache, fever, diabetes, indurations of the mammary glands, boils, stomach ache, intestinal disorder, worms, purgative, snake bite, dropsy and malaria, to mention but a few⁷. However, ethanolic leaf extract of *Momordica foetida* schum (Cucurbitaceae) has been reported to be teratogenic on the morphology of foetal-sprague dawley rats at the dose of 500 mg kg⁻¹ b.wt. This leaf extract was reported to reduce the crown rump length, trans-umbilical cord length compared to the control group and prevent implantation in Sprague-dawley rats⁸.

One of the plants of medicinal value is *Costus afer* belonging to Costaceae family. *Costus afer* is commonly called Gingerlily or bush sugar cane⁹. It is a monocot and a relatively tall, herbaceous, unbranched tropical plant with creeping rhizome. *Costus afer* is found in moist and shady forest of west and tropical Africa¹⁰. It is commonly used as a medicinal plant throughout tropical Africa in alleviation of inflammation, arthritis, rheumatism, hepatic disorders, hemorrhoids, cough, epileptic attack, miscarriages and helminthic, diuretics and laxatives as an antidote for poison¹¹⁻¹³. *Costus afer* also has antioxidant properties¹⁴ and as medicament to treat goat with retained placenta¹⁵. Several herbs taken by pregnant women cross the placenta and can

affect brain development. Exposure of these herbs to the fetus may have long-lasting implications for brain structure and function¹⁶. *Costus afer* is extensively used in our society for treatment of various ailments. There is tendency that it may be used intentionally or unintentionally by pregnant women, hence, the need for evaluation of its safety when used during pregnancy. Thus, this study is carried out to evaluate the teratogenic potential of ethanol leaf extract on the morphology and cerebral cortex of fetuses of albino Wistar rat.

MATERIALS AND METHODS

Study area: The study was carried out in the University of Calabar, Calabar, Nigeria and the duration of the research work was between February, 2017 to March, 2018.

Plant collection: The leaves of *Costus afer* were harvested from its stems manually from the Botanical garden of the University of Calabar, Calabar, Nigeria. The plant was identified and authenticated in the Botany Department, University of Calabar and with Voucher number: Herb/Bot/002. *Costus afer* leaves were washed and air-dried at room temperature thereafter, grinded into powdered form and used for the ethanolic extract preparation. The extraction was carried out in the Endocrine laboratory of Biochemistry Department, University of Calabar. The Ethical approval number (032AN20717) was given to carry out the research and all the guidelines of the Ethical committee were fully followed during the research.

Twenty four adult female Wistar rats weighing between 150 and 200 g bred in the animal house of the Department of Anatomy, University of Calabar was used for this study. The animals were grouped into 4 groups of 6 rats each and kept in separate wooden cage. The rats were fed with normal rat chow and water were provided *ad libitum* throughout the duration of the experiment. The females were caged overnight with sexually mature male rats of the same strain. The presence of sperm (tailed structures) in the vagina smears obtained the following morning confirmed coitus and the sperm positive day were designated as day zero of pregnancy. Group A served as control and received 0.5 mL of distilled water orally for same period with the experimental groups.

Group B, C and D served as experimental groups and were given 250, 500 and 1000 mg kg⁻¹ b.wt., of ethanolic leaf extract of *Costus afer*, respectively via oral route of administration from day 6-12 of gestation. Pregnancy was terminated on the 20th day of gestation and fetuses were harvested by uterectomy. The fetuses were blotted dry and

examined for gross malformations. Pups were weighed by Libror EB-330H sensitive balance, while tail length and crown-rump length were measured by using Vernier caliper. Data obtained from the experiment was statistically analyzed and expressed as Mean \pm Standard Deviation (SD) using SPSS and ANOVA followed by Scheffes *post hoc* test. Values of $p < 0.05$ were considered statistically significant. The heads were dissected through incisions along the dorsal aspect and the brains were removed, blotted dry with a filter paper. Histological examination of cerebral cortex using Hematoxylin and Eosin staining technique for the cerebral architectural organization was carried out.

RESULTS

The results indicates no observable malformation of the external structures. The structures were fully formed and developed. Table 1 shows fetuses from the groups of rats that were given 250, 500 and 1000 mg kg^{-1} of leaf extract of *Costus afer* demonstrated no significant ($p < 0.05$) reduction in fetal body weight with placenta, fetal body weight without placenta, crown-rump length and tail length measured when compared to the control and between the groups. However, a dose related reduction was observed between the groups.

Histological study of the cerebral cortex by using haematoxylin and eosin staining method showed marginal, cortical, intermediate, sub-ventricular and ventricular zones in the control group. Figure 1-4 shows the results of histological study carried out on the groups that received 250, 500 and 1000 mg kg^{-1} b.wt., of ethanolic leaf extract of *Costus afer*.

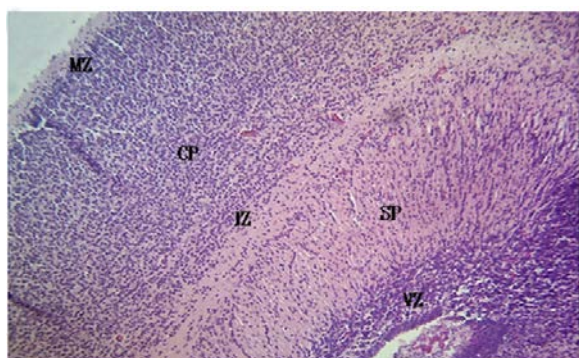


Fig. 1: Photomicrograph of a fetal cerebral cortex from control rats
MZ: Marginal zone, CZ: Cortical zone, IZ: Intermediate zone, SZ: Subventricular zone, VZ: Ventricular zone (H and E Mag $\times 100$)

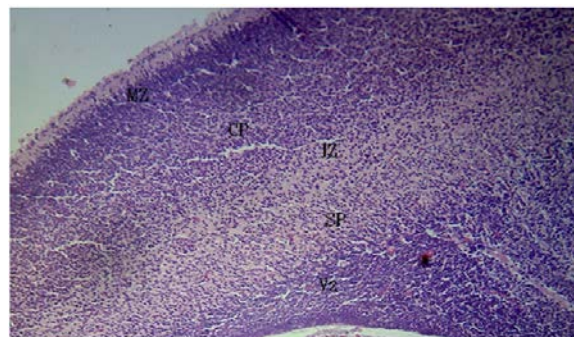


Fig. 2: Photomicrographs of cerebral cortex of fetuses whose maternal administration of ethanolic leaf extract of *Costus afer* was 250 mg kg^{-1} b.wt.
MZ: Marginal zone, CP: Cortical plate zone, IZ: Intermediate zone, SP: Subventricular plate zone, VZ: Ventricular zone (H and E $\times 100$ mag)

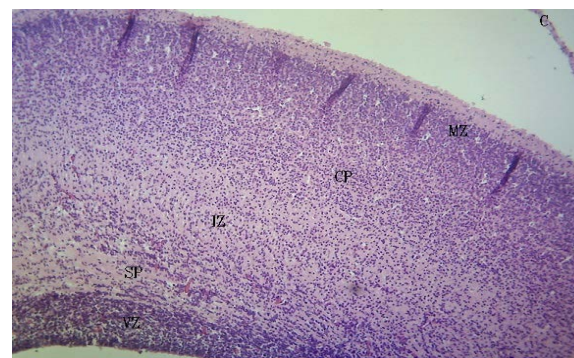


Fig. 3: Photomicrographs of cerebral cortex of fetuses whose maternal administration of ethanolic leaf extract *Costus afer* was 500 mg kg^{-1} b.wt.
MZ: Marginal zone, CP: Cortical plate zone, IZ: Intermediate zone, SP: Subventricular plate zone, VZ: Ventricular zone (H and E $\times 100$ mag)

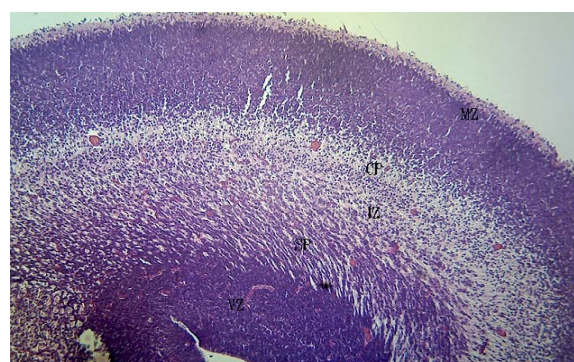


Fig. 4: Photomicrographs of cerebral cortex of fetuses whose maternal administration of ethanolic leaf extract of *Costus afer* was 1000 mg kg^{-1} b.wt.
MZ: Marginal zone, CP: Cortical plate zone, IZ: Intermediate zone, SP: Subventricular plate zone, VZ: Ventricular zone (H and E $\times 100$ mag)

Table 1: Effect of ethanol leaf extract of *Costus afer* on the fetal weight, fetal weight with placenta, crown-rump length and tail length

Parameters	Group A (control)	Group B (250 mg kg ⁻¹)	Group C (500 mg kg ⁻¹)	Group D (1000 mg kg ⁻¹)
Fetal body weight with placenta (g)	5.28±0.04	5.04±0.02	4.99±0.03	3.35±0.10
Fetal body weight without placenta (g)	4.70±0.02	4.39±0.01	4.39±0.01	2.73±0.08
Crown-rump length (cm)	3.53±0.02	3.50±0.00	3.49±0.01	3.16±0.04
Tail length (cm)	1.50±0.00	1.48±0.02	1.50±0.00	1.39±0.01

Results are presented as mean±SEM (p<0.05) when compared with control

DISCUSSION

In this study, the results indicated that administration of *Costus afer* to pregnant Wistar rats has no significant effect on the parameters studied. Several studies have demonstrated a correlation between intrauterine growth retardation and teratogenesis. Parameters used in examining intrauterine growth include; body weight^{17,18}, crown-rump length and tail length^{19,20}. The crown-rump length, tail length and placental weight of the treated groups were not significantly different when compared with the control rats. *Costus afer* has been reported to possess antioxidant properties^{14,21}, which may be responsible for the reduction in the formation of Reactive Oxygen Species (ROS) that could necessitate teratogen-induced fetal malformations. This study revealed that administration of 250, 500 and 1000 mg kg⁻¹ of ethanol leaf extract of *Costus afer* was not able to bridge development or induce malformation on the body weight, crown-rump length and tail length of fetuses of albino Wistar rats. However, the reduction observed in the group treated with 1000 mg kg⁻¹ is suggestive of dose related effect. The histological features of the treated cerebral cortex showed no reduction in the cell population, thickness of the zones and no disruption in the layers when compared to the control. Absence of any change in the histological features of the treated cerebral cortex may be due to non-teratogenic potential of *Costus afer*. This is in line with the report of the previous study²² which stated that the leaf extract of *Costus afer* are non-toxic to mice in sub-acute and acute dose of 5000 mg kg⁻¹. This study was limited to Haematoxylin and Eosin staining technique by using light microscope. However, there is need for further studies on the brain and demonstration of astrocytes using specific staining technique.

CONCLUSION

Results obtained in this rat model study with ethanolic leaf extract of *Costus afer* showed no observable malformation on the external structures of the fetuses and no evidence of alteration of the developing cerebral cortex of the treated groups when compared to the control group.

SIGNIFICANCE STATEMENT

This study suggest that *Costus afer* appears to be safe for use during pregnancy and probably has no toxic effect on the fetus. These knowledge will help researchers in the usage of this medicinal plant during gestation in the treatment of diabetes and its possible use in pharmaceutical industries can be explored.

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