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Research Article

INVESTIGATIONS OF METHANOLIC LEAF EXTRACT OF *COSTUS LUCANUSCIANUS* ON GESTATION IN ALBINO RATS

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ABSTRACT

Costus lucanuscianus, a medicinal plant locally called 'monkey sugar cane' in the Niger Delta region of Nigeria, is used in folk medicine for the treatment of diarrhoea, dysmenorrhea, headache and rheumatism. The paucity of information on the safety of *Costus lucanuscianus* uses in pregnancy prompted this study using methanolic leaf extract of this plant. Twenty successfully mated female rats were divided into four groups. Group A (Control) received 0.5ml/kg of 20% Tween 80 (vehicle), Group B (100 mg/kg of extract),Group C (200 mg/kg of extract), Group D (300 mg/kg of extract) by oral gavage from gestational day (GD) 6-19. On GD 20, laparotomy was performed. The foetal weights, foetal crown-rump length (FCRL), number of implants, resorptions, dead and live foetuses were recorded. The foetuses were examined for external and visceral malformations. *C. lucanuscianus* leaf extract did not cause abortion in the pregnant rats neither was any external nor visceral anomaly observed in the foetuses. The extract caused a significant decrease ($p<0.05$) in the percentage of live borne foetuses and significant increase ($p<0.05$) in the percentage of post-implantation deaths in rats of group D relative to Group A. Groups C and D showed a significant decrease ($p<0.05$) in FCRL relative to group A. From the study, it was concluded that methanolic leaf extract of *C. lucanuscianus* did not cause any obvious abortion in all the treated pregnant rats, rather it caused a dose dependent increase in the percentage of post-implantation deaths and decreased FCRL across the treated groups.

Keywords: *C. lucanuscianus*, Abortion, post-implantation death, foetal crump-rump length.

INTRODUCTION

The plant *Costus lucanuscianus* commonly known as spiral ginger is a perennial rhizomatous herb growing up to 3 metres tall (Fern, 2017). It is a common species in the forest zone of tropical Africa (Saliu and Fapohuda, 2016). *C. lucanuscianus* is a medicinal plant locally called 'monkey sugar cane' in the Niger Delta region of Nigeria. The juice from the leaves has a wide reputation in folk medicine for the treatment of diarrhoea, vomiting and dysmenorrhea (Gill, 1992).

The use of medicinal plants in pregnancy could have adverse effect on the developing foetus depending on the dose, route and duration of exposure. This has been observed with certain plant preparations or extracts (Enaibe *et al*., 2012, 2014; Ansah *et al*., 2016). There is however no evidence in literature that substantiates the safety of *Costus lucanuscianus* uses in pregnancy. This study is therefore designed to investigate the effect of *Costus lucanuscianus* leaf extract on pregnancy.

MATERIALS AND METHODS

Plant Material and Authentification

Fresh leaves of *Costus lucanusianus* were collected from the forest reserve of University of Port Harcourt, Nigeria. The plant was identified by Dr. I. Agbagwa of the department of Plant Science and Biotechnology, University of Port Harcourt, Rivers State, Nigeria, and a sample was deposited at the University of Port Harcourt Herbarium with reference number UPH/V/1212.

Preparation of plant Extract

After collection of the plant, the leaves were shade-dried at room temperature (32 – 35°C) to constant weight over a period of seven (7) days. The cold maceration extraction method of Cowan (1999) was used. Fifty grams of dried *Costus lucanusianus* leaves was weighed and grinded to fine powder and dissolved in 1000ml of seventy percent methanol inside a 2-liter conical flask. The flask was shaken vigorously at 30 minute intervals and left to stand for 72 hours at room temperature for effective extraction. The resultant mixture then was filtered with Watman's No. 1 filter paper and cotton wool to remove particles of plant sample. The clear solution obtained was concentrated with rotary evaporator at 45°C under low pressure and later transferred to evaporating dish over a steam bath. The solid dried powder obtained was stored in sterile pre-weighed screw capped bottles and labelled accordingly. The extract was now stored at room temperature.

ANIMALS

Twenty mature female albino rats weighing an average of 210g, procured from the Animal House of Department of Pharmacology, College of Health Sciences, University of Port Harcourt, Nigeria were used for the study. The rats were acclimatized for two (2) weeks before commencing the study. They were fed *ad libitum* with commercially sourced feed (Top Feeds Nigeria Limited) and supplied with clean drinking water all through the study.

Experimental Procedure

Following acclimatization, one female was paired with a male in a cage overnight. Mating was confirmed the following day by the presence of sperm cells in vaginal smear or presence of vaginal plug and designated gestational day (GD) 0. The mated females were randomly assigned to four (4) groups of five animals each for treatment as follows:

Group A (Control) received 0.5ml/kg body weight of 20% Tween 80 (vehicle).

Group B received 100 mg/kg body weight of the extract

Group C received 200 mg/kg body weight of the extract

Group D received 300 mg/kg body weight of the extract

Administration of extract and vehicle was by oral gavage daily from gestational day (GD) 6 – 19. Animal's weight was taken daily and the dose adjusted accordingly. On gestational day (GD) 20, the animals were anaesthetized. Laparotomy was performed and the uterine horns were removed. The number of implants, resorptions, dead and live foetuses were recorded. The foetal weights and Foetal crown-rump length were also taken. The foetuses were examined for macroscopic external malformations. Some of them were fixed in Bouin's solution for visceral examination.

Statistical Analysis

Statistical analysis was done using SPSS 21. All values were expressed as mean ± SEM and data were assessed by one-way ANOVA followed by the LSD post-test. The significance level was set at $p<0.05$.

RESULTS

Table 1 summarizes the effect of the extract on rat foetuses obtained from dams exposed to gestation day (GD) 6-19. The results showed that *Costus lucanuscianus* leaf extract had no significant effect on the foetal weights and total number of uterine implant ($p>0.05$) in all the treated groups. Although there was no significant difference ($p>0.05$) in the percentage of live borne foetuses and post-implantation deaths in rats in groups B and C when compared with group A, the result showed a significant decrease ($p<0.05$) in the percentage of live borne foetuses and significant increase ($p<0.05$) in the percentage of post-implantation deaths respectively in rats of group D relative to the control (Group A). Groups C and D showed a significant decrease in foetal crown-rump length relative to group A.

Table 1: Effects of methanolic leaf extract of *Costus lucanuscianus* on the rat foetuses of exposed dams (GD 6-19)

Parameters	GROUP A	GROUP B	GROUP C	GROUP D
Total Uterine Implants	7.25 ± 0.75	7.00 ± 0.91	9.50 ± 0.50	6.00 ± 0.75
Post-Implantation Deaths (%)	0.00 ± 0.00	5.56 ± 5.56	12.50 ± 7.22	26.46 ± 13.12*
Live-Borne Foetuses (%)	100.00 ± 0.00	94.45 ± 5.56	88.75 ± 6.57	73.54 ± 13.12*
Foetal Weight (g)	5.82 ± 0.35	5.72 ± 0.42	4.77 ± 0.75	4.94 ± 0.64
Foetal crown-rump length (cm)	4.30 ± 0.10	4.14 ± 0.06	3.91 ± 0.13*	3.97 ± 0.14*

Group A =control, Group B =100mg/kg *C.lucanuscianus* extract, Group C =200mg/kg *C.lucanuscianus* extract, Group D =300mg/kg *C.lucanuscianus* extract.

Post-Implantation Deaths (%) = [(Total no. of implantation – Total no. of Live-Borne Foetuses)/ Total no. of implantation] × 100

Values expressed in Mean ± SEM.

* indicates a significant variation ($P<0.05$) when compared to the control (Group A)

Furthermore, No clinical sign associated with maternal toxicity such as maternal death, locomotor alterations, diarrhoea or piloerection was observed in the dams in all the treated groups. No external and visceral anomaly in the live borne foetuses was observed in all the treated groups. There was no abortion recorded in all the treated groups.

DISCUSSION

Recent studies have shown that a wide range of medicinal plants used as herbal remedies may have adverse effects in both male and female animals including infertility, increased foetal death, teratogenesis and abortion (Akpantah *et al*., 2005; Sripriya *et al*., 2011; Eze, 2012; Ekhator and Osifo, 2015; Odirichukwu, 2015; Adeleke *et al*., 2016; Ansah *et al*., 2016).

In the present study, oral administration of methanolic leaf extract of *Costus lucanuscianus* at the doses 100, 200 and 300 mg/kg from gestation day (GD) 6-19 did not cause any obvious abortion in the pregnant rats; rather it produced a dose dependent increase in the percentage of post-implantation deaths and decreased foetal crown-rump length across the treated groups. No external and visceral anomalies were observed in the live borne foetuses. A similar finding of dose-dependent increase in post implantation deaths has been reported in rats dosed with 100, 200 and 500 mg/kg of acetone extract of *Ocimum gratissimum* stem on days 1-5 of pregnancy (Sripriya *et al.,* 2011). The post implantation deaths recorded suggests that interruption of pregnancy occurred after implantation (Elbetieha *et al*., 2000). Moreover, foetal crown-rump length comparison which is an important parameter that indicates intrauterine growth retardation in response to the foetal exposure to a toxicant (Raees *et al*., 2010) suggests that *Costus lucanuscianus* could be harmful to the developing foetus.

On the contrary, our result disagrees with the findings by Anaga et al. (2004), who reported that methanolic extract of *Costus afer*, a closely related species to *Costus lucanusianus* (Aweke, 2008) when administered intraperitoneally to pregnant rats at the doses of 50, 100 and 150 mg/kg, caused a 100% abortion in the 3rd trimester of pregnancy. The disparity in the result of the present study and that of Anaga *et al*. (2004) could be due to the route of administration used in the studies and the species variation. Foungbe *et al*. (1987) demonstrated that *Costus lucanusianus* exhibits uterine relaxant activity which could be the basis for its folkloric use in Ivory Coast for treating impending abortion. According to Owolabi et al. (2010), the aqueous extract of *Costus lucanusianus* leaves has an oxytocic effect on isolated non-pregnant rat uterus contrary to its acclaimed folkloric use in the treatment of threatened abortion and dysmenorrhea, where a relaxant rather than contractile effect on the uterus was expected.

CONCLUSION

This study suggests that *Costus lucanusianus* leaf extract could be detrimental to gestation as was indicated in the dose dependent increase in the percentage of post-implantation deaths and decreased foetal crown-rump length across the treated groups. It is however recommended that further research should be carried out to isolate and identify the mechanism of toxicity and the toxic principle(s) associated with the crude leaf extract of this plant.

REFERENCES

1. Fern K. Useful Tropical plants Data base 2014. http://www.prota.org.
2. Saliu AJ and Fapohunda O. The Antihyperglycemic, Hepatoprotective and Renoprotective potentials of the aqueous extract of Costus lucanuscianus on Streptozotocin-induced diabetic rats. J Appl Life Sci Int. 2016;4(2):1-10.
3. Gill LS. Carica papaya L. In Ethnomedicinal Uses of Plants in Nigeria. UNIBEN Press, Benin City, 1992;57–58.
4. Enaibe BU, Omotoso GO and Ayanwale OO. Histological evaluation of the embryotoxic and neurotoxic effects of Mangifera indica in prenatally exposed Wistar rats. J Exp Clin Anat. 2012;11 (2): 8-13.
5. Enaibe BU, Omotoso GO, Olajide OJ, Lewu SF and Adeyemi SO. Morphological evaluation of the superior colliculus of young Wistar rats following prenatal exposure to Caricapapaya leaf extract. J Expt and Clin Anat. 2014;13(2):29-33.
6. Ansah C, Appiah JA, Mensah KB and Mante PK. Aqueous leaf extract of Carica papaya (Caricaceae) linn. causes liver injury and reduced fertility in rats. Int J Pharm Pharm Sci. 2016; 8(2):261-265.
7. Cowan MM. Antimalarial activity. Clin Microbiol Reviews. 1999;12(4):564-582.
8. Akpantah AO, Oremosu A, Noronha AA, Ekanem TB and Okanlawon AO. Effects of Garcinia kola seed extract on ovulation, oestrus cycle and fetal development in cyclic female Sprague-

Dawley rats. Nig J Physio Sci. 2005;20(1-2):58-62.
9. Sripriya S, Yuvaraj G, Nema RK, kumar VM and Deecaraman M. Evaluation of Antifertility activity from Stem Part of Ocimum gratissimum in Acetone extracts. Int J Pharm and Clin Res. 2011;3(2):41-44.
10. Eze KN. Antifertility effects of ethanolic extract of Xylopia aethiopica on male reproductive organ of wistar rats. American J Med and Med Sci. 2012;2(1):12-15.
11. Ekhator CN and Osifo UC. Abortificient efficacy of Moringa oleifera leave: An Experimental study on Adult Female Wistar Rats. American J Biol and Life Sci. 2015;3(6):269-272.
12. Odirichukwu EO. Effects of Aqueous Methanolic Extract of Unripe Carica Papaya Fruit on Midterm Pregnancy in Wistar Albino Rats J Vet Adv. 2015;5(6):962-967. DOI: 10.5455/jva.20150523091721.
13. Adeleke OK, Sangoyomi T and Moody JO. Effect of Cnidoscolous aconitifolius leaf extract on sperm characteristics and reproductive hormones of male rats. Int J Phytomed. 2016;8(2):228-237.
14. Elbetieha A, Oran SA, Alkofahi A, Darmani H and Raies AM. Fetotoxic potentials of Globularia alypum (Globulariaceae) in rats. J. Ethanpharmacol. 2000;72:215-219.
15. Raees, KA and Khawaja RA. Pregnancy and foetal correlations of cypermethrin in mice (mus musculus). Biologia (Pakistan). 2010;56(1&2):39-54.
16. Anaga AO, Njoku CJ, Ekejiuba ES, Esiaka MN and Asuzu IU. Investigation of the methanolic leaf extract of Costus afer Ker for pharmacological activities in vitro and in vivo. Phytomed. 2004;11: 242-248.
17. Aweke G. Costus lucanuscianus. In Medicinal plants 1Edited by Schmelzer GH, Schmelzer GH and Gurib-Fakin A. Prota. 2008;194-196.
18. Foungbe S, Sawadogo D and Delume C. Experimental study of the uterine-relaxant activity of Alstonia boonei (Apocyanaceae) and Costus lucanuscianus (Zingiberaceae) traditionally used as anti-abortion agent in the Ivory Coast. Ann Pharm Fr. 1987;45:373.
19. Owolabi OJ, Omogbai E and Falodun A. Oxytocic effects of the aqueous leaf extract of Costus lucanuscianus - Family Costaceae on isolated non-pregnant rat uterus. Pak J Pharm Sci. 2010;23(2):207-211.