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IN VITRO INHIBITORY EVALUATION OF *COLA HISPIDA*, BRENNAN AND KEAY (STERCULIACEAE) ON THE ISOLATED RAT UTERUS

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ABSTRACT

The ethanolic extract of the leaves of *Cola hispida* (S*terculiaceae*) a medicinal plant used locally for the treatment of threatened abortion was screened for uterine activity. Isolated rat uteri from rats pre-treated with diethylstiboesterol were challenged with the extract to determine the activities of the extract on the uterus. The effect of the extract on oxytocin and acetycholine-induced contraction were also studied. The extract produced significant inhibition of spontaneous contraction of the uterus and also significantly inhibited both oxytocin and acetylcholine-induced contraction ($P<0.05$). This inhibition was dose–dependent. Doses less than 0.4 mg/ml {final bath concentration} of the extract had no observable inhibitory effect. Its relaxant activity was 60 and 50 % of the inhibitory effects produced by salbutamol (0.002 µg/ml) and atropine (0.02 µg/ml) on oxytocin and acetylcholine induced contractions respectively. It was thus concluded that the leaves of *Cola hispida* possesses tocolytic properties.

Keywords: *Cola hispida*, rat uterus, tocolytic activity, leaves extract.

INTRODUCTION

A good proportion of the world population particularly those living in developing countries like Nigeria depend mostly on herbal medicine for their health needs (Sofowora, 1996). This is due to the following factors: easy accessibility, low cost and ancestral experience. In developing countries, it is important to develop systems through which effective health care can be made both accessible and acceptable to the people (Sofowora, 1996).

Cola hispida, Brennan and Keay, family: Sterculiaceae is a small forest tree, growing up to 4-12 m high, occasionally up to 20 m, with a low crown of arching branches. Bark is pale or dark-grey in color (Gill, 1992). Its distribution is more in the eastern and northern parts of Nigeria. It is also found in Cameroon, Gabon and Zaire. A decoction of the leaf is locally used to prevent premature labour (Keay, 1989). Premature labor is the onset of labor before the expected date resulting in delivery of an immature infant. Preterm birth is a major cause of neonatal mortality and morbidity in the developed world. The perfect tocolytic that is uniformly effective with complete fetomaternal safety does not exist. Tocolytic agents differ in cost, utero-specificity, safety and efficacy. The main three agents that are used worldwide are beta-agonists, calcium channel blockers and vasopressin /oxytocin receptor antagonists. (Lyndrup and Lamant, 2007). A review of the literature revealed that previous work has not been conducted on the uterine activity of the leaves of *Cola hispida*. Hence the need for this work to give credence to its folkloric use in the treatment of premature labour.

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MATERIALS AND METHODS

Plant material

The leaves of *Cola hispida* were collected in Benin City, Edo State, Nigeria, in 2007. The botanical identity of the plant and its leaves were authenticated by Prof. Idu, Department of Botany, University of Benin, Nigeria. Immediately after collection, leaves were air dried for nine days. The dried leaves were pulverized into a fine powder using impact mill, weighed and kept for further analysis.

Extraction

The powdered material was extracted with the aid of a soxhlet apparatus, using absolute ethanol (2.5 litres) as the solvent. The extract was filtered and the filtrate evaporated to dryness with the aid of a rotary evaporator attached to a vacuum pump at 40 ºC. The concentrated extract was stored in air tight containers, labelled and refrigerated at 4 ºC prior to use.

Drugs and Chemicals

The following drugs were used: diethylstilbestrol (Merck), Oxytocin Injection (Laborate Pharmaceuticals, India), Acetylcholine (Sigma Chemical, USA), extract of *Cola hispida*, Dimethysulphoxide, Absolute Ethanol (Sigma Chemical, USA), Salbutamol (Glaxosmithkline) and Atropine (Sisbu Xierkang Pharm Co.).

Animals

Female non-pregnant Wistar rats weighing 130-150g were obtained from the Animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Edo State, Nigeria. The animals were maintained on a standard diet (Ladokun feeds, Ibadan, Oyo State) and had access to food and water *ad libitum.* Animals were housed four in a cage with a twelve hour light-dark cycle. [All experiments conformed to acceptable protocols for use of animals in experiment].

Animal preparation

Female non-pregnant Wistar rats were pretreated intraperitoneally with 0.2 mg/kg of Diethylstilbestrol 24h prior to the actual experiment (Veale *et al.*, 1989). The rats were killed by cervical dislocation and exanguinations. The abdomen was opened and the two horns of the uterus carefully isolated, freed of mesenteric fat and a 1cm piece was mounted in a 50 ml organ bath containing De Jalon physiological salt solution having the following chemical composition: NaCL, 9 g/l, $NaHCO_3$, 0.5 g/l, D-glucose, 0.5 g/l, KCL, 0.402 g/l, $CaCL_2.2H_20$, 0.08 g/l.

The tissue was aerated with 95 % oxygen 5 % carbon (IV) oxide and temperature maintained at 37 ^oC, with a PH of 7.4. The spontaneous contraction of the uterus was recorded with FT 03 transducer connected to an Ugo Basile recorder (7075). The transducer was previously calibrated to establish a relationship between the force applied to the transducer and the gauge deflection (500mg).

The tissue was put under 500 mg tension and was allowed to equilibrate for 30 minutes before the commencement of the experiment. The effect of the extract on the spontaneous contraction of the tissue was determined. Responses to different doses of oxytocin ($4x10^{-5}$ I.U/ml to $8x10^{-2}$ I.U /ml: Final organ bath concentration) and in the presence of the extract were obtained. Responses to acetylcholine (0.04 to 40µg/ml) alone and in the presence of the extract were also determined. The effects of two positive controls (salbutamol and atropine) were also determined.

Statistical analysis

All results are expressed as the mean of five experiments ± SEM (standard error of mean) and continous line graph. The data were analyzed statistically by student's t-test using Graphpad instat version 2.05a. The level of significance was $P<0.05$

RESULTS

From the experiment, it was observed that the extract at a dose of 50 mg/ml (0.67 mg/ml: final organ bath concentration) inhibited the spontaneous contraction of the uterus. Oxytocin at $3.2x10^{-4}$ I.U/ml (final organ bath concentration) alone gave a mean response of 54.88 % of the maximum response, while the same concentration in the presence of the extract gave mean response of 16.03 %. At oxytocin concentrations less than $3.2x10^{-4}$ iu/ml there was no observable response in the presence of the extract (Fig. 1). The maximum response obtained in the presence of the extract for oxytocin-induced contraction was 75 %.

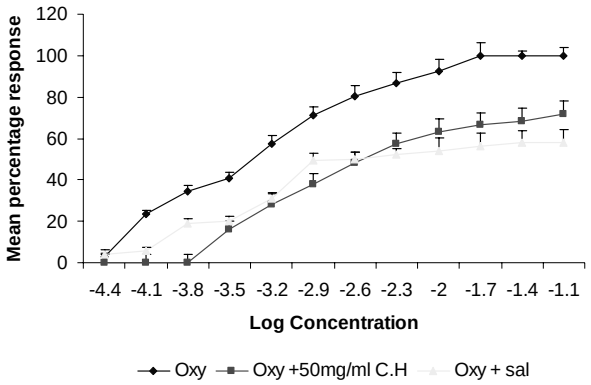

Fig. 1. Inhibitory activity of the ethanolic extract of *Cola hispida* on oxytocin induced contraction of the isolated rat uterus.

Values are mean percentage responses ± SEM, (n = 5 animals), C.H stands for the ethanolic extract of *Cola hispida.*

The extract significantly ($P<0.05$) reduced the percentage response due to oxytocin.

For acetylcholine-induced contraction, 0.16 µg/ml of acetylcholine gave 70.9% of maximum response while the same concentration gave 28.8% in the presence of the extract (Fig. 2).

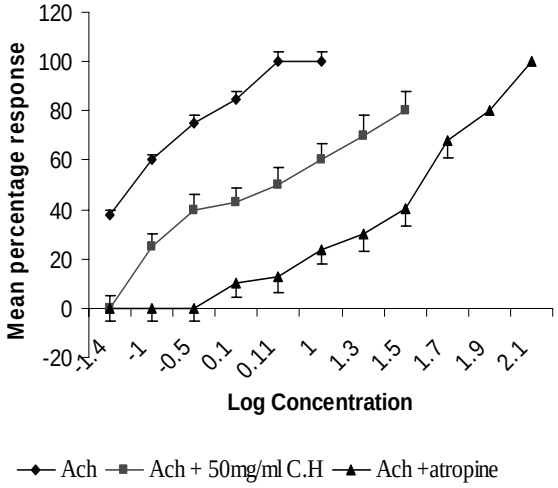

Fig. 2. Inhibitory activity of the ethanolic extract of *Cola hispida* on acetylcholine induced contraction of the isolated rat uterus.

Values are mean percentage responses ± SEM.

(n = 5 animals).
C.H stands for the ethanolic extract of *Cola hispida.*
The extract significantly (P<0.05) reduced the percentage response due to oxytocin.

The results in fig 1 also shows a comparative inhibitory activity produced by the extract and salbutamol which is used clinically in the treatment of threatened abortion in gravid uterus, while figure 2 shows a comparative inhibitory activity produced by the extract and atropine on acetylcholine induced contraction.

The activity of the extract compares well with salbutamol and atropine, two positive controls that significantly (p<0.0001) relaxed the uterus (Fig.1 and 2 respectively).

DISCUSSION

Our results showed that the extract shifted the dose-response curve of oxytocin to the right and also depressed the maximum response. In the case of Acetylcholine, while it shifted the dose-response to the right, the maximum response was achieved.

It is therefore possible that in the case of Oxytocin, the extract is acting as a physiologic antagonist where Oxytocin is causing contraction and the extract is relaxing the uterus.

But in the case of acetylcholine, the fact that the maximum response was obtained indicates that the extract was competing with acetylcholine receptors and when a high concentration of acetylcholine was added, the maximum response was achieved. Thus we could say that the extract probably act through the muscarinic receptor (Newal *et al.,* 1986).

In addition, interestingly the extract was found to inhibit the normal spontaneous contraction of the uterus.

The results suggest that the ethanolic extract of *Cola hispida* has a potential tocolytic effect that can be explored for therapeutic advantage as an alternative treatment for threatened abortion and dysmenorrhoea.

CONCLUSION

The findings of our study indicate that the ethanolic extract of *Cola hispida* possess inhibitory activity on the uterine smooth muscles in non-pregnant rats, which is consistent with the literature report of its use in the treatment of preterm labour.

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REFERENCES

Gill, LS. 1992. Ethnomedical uses of plants in Nigeria. Uniben Press, Benin City. pp.143.

Keay, RWJ. 1989. Trees of Nigeria. Clarendon Press Oxford. pp. 475.

Lyndrup, J. and Lamont, RF. 2007. The Choice of a Tocolytic for the Treatment of Preterm Labour: A Clinical Evaluation of Nifedipine Versus Atosiban., Expert Opin Investig. Drugs. 16(6):843-853.

Newal, CA., Anderson, LA. and Phillipson, JD. 1986. Herbal medicine, a guide for health care professionals. Pharmaceutical press. 291-296.

Sofowora, A. 1996. Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria. 100-107.

Veale, DJH., Oliver, DW., Arangics, N.S. and Furman KJ. 1989. Preliminary isolated organ studies using an aqueous extract of *Clivia miniata* leaves. Journal of Ethnopharmacology. 37:341-346.