

NATURAL DRUGS

EVALUATION OF TOCOLYTIC ACTIVITY OF ETHANOL EXTRACT OF THE STEM BARK OF *FICUS CAPENSIS* Thunb. (MORACEAE)OMONKHELIN J. OWOLABI^{a*}, ZULEKHAI A. NWORGU^a, ABIODUN FALODUN^b,
BUHIYAMIN A. AYINDE^c and CHARLES N. NWAKO^d.^aDepartment of Pharmacology and Toxicology, ^bDepartment of Pharmaceutical Chemistry, ^cDepartment of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City, Edo State, Nigeria. ^dFederal Medical Centre, Owerri, Imo State, Nigeria

Abstract: *Ficus capensis*, a wild fig tree is used in herbal medicines to treat pregnancy related ailment and most especially as a remedy for threatened abortion. The purpose of this study was to investigate this claim with view to validating scientifically the ethno-medicinal usage. The ethanolic extract obtained by maceration technique was subjected to pharmacological testing *in vitro* on a piece of isolated rat uterus previously pretreated with stilbestrol, suspended in De Jalon at 37°C. Concentrations used were 40 mg/mL and 80 mg/mL. The higher concentration (80 mg/mL) significantly ($p < 0.05$) exerted smooth muscle relaxant activity on the uterus (a reduction of oxytocin, ergometrin and acetylcholine induced contractions as well as an increase of the EC₅₀ was observed for all the agonists tested in the presence of the extract). Evaluation of the data also indicated that the effect of the 40 mg/mL concentration was statistically insignificant, although a lowering of the dose response curve was observed for oxytocin, acetylcholine and ergometrin. Its relaxant activity at 80 mg/mL was 40 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. The results indicate the presence of active principles in the bark extract of *Ficus capensis* which may be responsible for some of the applications in traditional medicines as an anti-abortifacient and as a remedy against threatened abortion.

Keywords: bark extract, *Ficus capensis*, tocolytic activity, rat uterus

Herbal medicine has its origin from ancient culture including those of the Egyptians, American-Indians and Chinese. It involves the medicinal use of plants to treat diseases and enhance general health and well being (1)

Ficus capensis is classified under the Moraceae species. The Moraceae family has about 53 genera and 1400 species. It is a wild fig deciduous or evergreen tree which can grow to 70 ft. tall, but usually attains about 40 feet. The fruits are round, approximately 2-3 cm in diameter (2).

The following compounds constitutes the chemical composition of the plant which are responsible for its ethnomedicinal uses. They include xantholoxin, β-amyrin and α-amyrin. Others are: campesterol, stigmaterol, its 3-β-O-glucoside and 4,5,7-trihydroxyflavan-3-ol (3). The plant is abundant in the tropics and is widely used in Southern, Central and West Africa as a herbal remedy for various ailments such as diarrhoea, rheumatism and threatened abortion. Various parts

of the tree have been reported to possess medicinal properties. The stem bark in particular is quite often used in folk medicine for the treatment of threatened abortion (4).

The uterus shares a number of similarities with the stomach, both belong to the group of smooth muscles that are spontaneously active and both maintain force as their volume increases and to expel its content (5). Data on the effect of *Ficus capensis* on the contraction or relaxation of the uterine smooth muscle is lacking.

In the present study, we evaluated the ethanolic extract of the stem bark for possible activity on the isolated uterus of non-pregnant rats based on its use as a remedy for threatened abortion

EXPERIMENTAL

Plant material

The barks of *Ficus capensis* (Thunb.) were collected in Owerri, Imo State, Nigeria, in April 2008.

* Corresponding author: e-mail: josphineomo@yahoo.com; phone no.: 08034120318.

The botanical identity of the plant and its bark were authenticated by Mr Sunny Nweke, of the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Nigeria. Immediately after collection, barks were cut into small pieces and air dried. The dried barks were pulverized into a fine powder using impact mill, weighed and kept for further analysis.

Extraction

The powdered material (57 g) was macerated with absolute alcohol (0.6 L) and left for 72 h. The mixture was stirred at six-hourly intervals using a sterile glass rod. The extract was filtered and the filtrate was evaporated to dryness at 40°C with the aid of a rotary evaporator attached to a vacuum pump. The concentrated extract was stored in air tight containers, labelled and refrigerated at -4°C prior to use.

Phytochemical screening

Qualitative tests for the presence of plant secondary metabolites such as carbohydrates, alkaloids, tannins, flavonoids, saponins and glycosides were carried out on the plant using standard procedures (1).

Drugs and chemicals

The following drugs were used: diethylstilbestrol (Merck), Oxytocin (Rotex Medica),

Salbutamol, Ergometrin (GlaxoSmithKline), Acetylcholine (Sigma Aldrich), and Atropine (Sisbu Xierkang Pharm Co. Ltd.), Absolute ethanol (Sigma Aldrich).

Laboratory animals

Female non-pregnant Wistar rats weighing 150-180 g were obtained from the Animal house of the Department of Pharmacology and Toxicology,

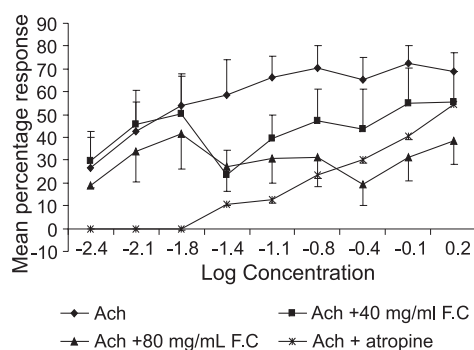


Figure 2. Inhibitory activity of the ethanolic stem bark extract of *Ficus capensis* on acetylcholine induced contraction of the isolated rat uterus. Values are the mean percentage responses \pm SEM. (n = 4 – 6 animals). F.C stands for the ethanolic extract of the stem bark of *Ficus capensis*. Ach – acetylcholine. The extract at 80 mg/mL significantly ($p < 0.05$) reduced the percentage response due to acetylcholine. The effect of the 40 mg/mL concentration was insignificant.

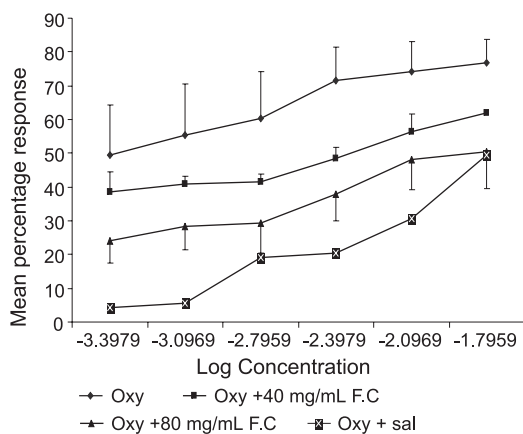


Figure 1. Inhibitory activity of the ethanolic stem bark extract of *Ficus capensis* on oxytocin induced contraction of the isolated rat uterus. Values are the mean percentage responses \pm SEM. (n = 4 – 6 animals). F.C stands for the ethanolic extract of the stem bark of *Ficus capensis*. Oxy – Oxytocin, Sal – Salbutamol. The extract at 80 mg/mL significantly ($p < 0.05$) reduced the percentage response due to oxytocin; however, the extract's effect at 40 mg/mL was insignificant.

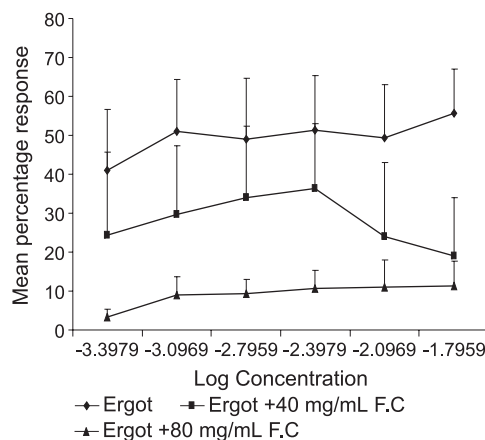


Figure 3. Inhibitory activity of the ethanolic stem bark extract of *Ficus capensis* on ergometrin induced contraction of the isolated rat uterus. Values are the mean percentage responses \pm SEM. (n = 4 – 6 animals). F.C stands for the ethanolic extract of the stem bark of *Ficus capensis*. Ergot – Ergometrin. The extract at 80 mg/mL significantly ($p < 0.05$) reduced the percentage response due to ergometrin. The effect of the 40 mg/mL concentration was insignificant.

Faculty of Pharmacy, University of Benin, Benin City, Edo State, Nigeria. The animals were maintained on a standard diet (Ewu feeds, Edo State, Nigeria) and had access to food and water *ad libitum*. Animals were housed four in a cage with a 12 h 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 24 h prior to the actual experiment (6). The rats were killed by cervical dislocation and exsanguinations. The abdomen was opened and the two horns of the uterus carefully isolated, freed of mesenteric fat and a 1 cm piece was mounted in a 50 mL organ bath containing De Jalon physiological salt solution having the following chemical composition: NaCl – 9 g/L, NaHCO₃ – 0.5 g/L, D-glucose – 0.5 g/L, KCl – 0.402 g/L, CaCl₂ × 2 H₂O – 0.08 g/L. The tissue was aerated with 95% oxygen and 5% carbon dioxide and temperature was maintained at 37°C, with a pH of 7.4. The spontaneous contraction of the uterus was recorded with 7003-B 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 (700 mg to 1.5 inches). The tissue was allowed to equilibrate for 30 min before the start of the experiment and placed under tension of 500 mg.

The dose-response curves of oxytocin, ergometrin and acetylcholine induced contractions were first obtained, the effect of the ethanolic extract (40 and 80 mg/mL) on the above dose response curves and that of two positive controls (salbutamol and atropine) were also determined.

Statistical analysis

All results are expressed as the mean of four to six experiments ± SEM. The statistical package used was SAS, 1994 Users guide, Version 8.2. SAS Institute Inc., Cary, NC, USA. The statistical significance ($p < 0.05$) of differences between the means was assessed by an analysis of variance (ANOVA) followed by Duncan's multiple range test.

RESULTS AND DISCUSSION

The plant extraction gave a yield of 20.4%.

Phytochemistry

The plant was observed to contain saponins, cardiac glycosides, tannins and flavanoids with traces of alkaloids and anthracene derivatives.

Pharmacological screening

The results showed that various concentrations of oxytocin (0.2 to 0.8 mL of 0.1 and 1.0 I.U.) and ergometrin (0.2 to 0.8 mL of 10 and 100 µg/mL) produced a significant contraction of the rat uterus, with maximum response being produced at 0.2 mL of 1.0 I.U. for oxytocin and 0.4 mL of 10 µg/mL for ergometrin). This was also noted for acetylcholine (0.2 to 0.8 mL of 1.0, 10.0 and 100.0 µg/mL) which also produced significant contraction of the uterus with the maximum produced by 0.2 mL or 100.0 µg/mL.

Evaluation of the data indicates that there was a significant ($p < 0.05$) dose-dependent reduction in oxytocin, ergometrin and acetylcholine induced contractions by the ethanolic extract at 80 mg/mL concentration tested (Figures 1-3).

The results in Figure 1 also show 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. Figure 3 shows the inhibitory activity produced by the extract against ergometrin induced contraction.

The activity of the extract (80 mg/mL) corresponds well with salbutamol and atropine, two positive controls that significantly ($p < 0.0001$) relaxed the uterus (Fig. 1 and 2, respectively), producing about 50% and 60% of the inhibitory effects of salbutamol and atropine, respectively.

The uterus is spontaneously active, which means that, with or without any nervous/hormonal stimulation, a piece of isolated, pregnant or non-pregnant, uterus will produce regular spontaneous contractions (7).

Our results showed that the ethanolic extract at 80 mg/mL produced significant inhibition of oxytocin, ergometrin and acetylcholine induced contractions of the uterine smooth muscle in non-pregnant rats. An increase in the EC₅₀ was also observed in the presence of the extract.

For oxytocin, ergometrin and acetylcholine an increase of 10, 96 and 50% in the EC₅₀ was observed for the 40 mg/mL concentration, respectively, while an increase of 50, 99.5 and 60% was observed for the 80 mg/mL concentration for oxytocin, ergometrin and acetylcholine, respectively. A shift of the dose response curve in the presence of the extract (80 mg/mL) was also observed.

The effect of the lower concentration (40 mg/mL) was, however, statistically insignificant, although there was a slight shift of the dose response curve to the right.

A blockade of acetylcholine induced contraction by the extract was observed. This blockade is similar to that produced by atropine.

It can thus be inferred that the extract might act *via* muscarinic receptors since there was significant blockade at a higher concentration of the extract.

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

CONCLUSION

The findings of our study indicate that the ethanolic extract of *Ficus capensis* stem bark possesses 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 threatened abortion).

It's envisaged that the active ingredients (compounds) will have a potential for being added to the present list of tocolytic agents used clinically. To improve the safety of this traditional herbal remedy, additional research is needed to define the stability and bioactivity of this product (8). Therefore, further

studies are needed for the isolation and characterization of the active constituents.

REFERENCES

1. Sofowora A.: Medicinal plants and traditional medicine in Africa, pp. 100-107. Spectrum Books Ltd. Ibadan, Nigeria 1996.
2. Arnold T.H., DeWet B.C.: Plants of southern Africa: names and distribution, Mem. Bot. Surv. S. Africa no. 62. (Pl SAfr) (1993).
3. Hassan A., Almahy D.: Pak. J. Soc. Sci. 3, 751 (2005).
4. Berg C.C.: *Kirkia* 13, 257 (1990).
5. Bulletti C., De Ziegler D., Polli V., Diotallevi L., Del Ferro E., Flamigni C.: Human Reprod. 15, 81 (2000).
6. Veale D.J.H., Oliver D.W., Arangics N.S., Furman K.J.: J. Ethnopharmacol. 37, 341 (1989).
7. Brenninkmeijer C.B., Price S.A., Lopez B.A.: J. Endocrinol., 162, 401 (1999).
8. Perharic L., Shaw D., Colbridge M.: Drug. Saf. 11, 284 (1994).

Received: 22. 08. 2008