VOL. 7, NO. 6, JUNE 2012 ISSN 1990-6145
ARPN Journal of Agricultural and Biological Science


www.arpnjournals.com

POTENTIALS OF ETHANOL EXTRACTS OF INFECTED FLORETS OF *Panicum maximum* JACQ IN EVOKING UTERINE CONTRACTION IN SPRAGUE-DAWLEY RATS

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ABSTRACT

The contractility effects of aqueous, ethanol and chloroform crude extracts (0.0312-2.0mg/ml final bath concentration (FBC) and purified ethanol fractions (0.001-3.0 mg/ml final bath concentration) of infected *Panicum maximum* floret were evaluated on primed isolated rat uterus in the presence of ergometrine (10^{-5}-10^{-2} mol/L). The crude aqueous, ethanol and chloroform extracts produced dose-dependent contraction of the uterus with ethanol extract being more potent than others, with the least dose achieving 77% contraction at FBC of 0.062mg/ml. Through the bioassay-guided fractionation, ethanol fraction produced a similar pattern of contraction with standard drug (ergometrine). Out of the three compounds detected in the ethanol fraction on further purification, the compound with R_f value of 0.20 (identified to be alkaloid) was active. These findings suggest the possible use of this compound as a new type of uterotonic agent for uterine contraction.

Keywords: *Panicum maximum*, contractility, extracts ergometrine, fractionation, alkaloid, uterine contraction, rats.

INTRODUCTION

Bleeding after childbirth is a leading cause of maternal mortality in developed and developing countries. Statistics from World Health Organization suggests that 25% of maternal death are due to postpartum haemorrhage (PPH) accounting for more than 100, 000 maternal death per year (Abouzahr, 1998). Uterine atony (inability or failure of the uterus to contract after childbirth) and incomplete expulsion of placenta are the major causes of bleeding after childbirth (Jackson *et al*., 2002; Sheiner *et al*., 2005).

The use of plants to facilitate birth appears to be a common practice among the traditional healers. Agents that stimulate uterine contraction are classified as oxytocic and are employed clinically for the induction and augmentation of labour as well as in the management of the third stage of labor. Some of these agents/ toxic substances have been reported to be produced during infection process of some fungi on grasses (Vandougen *et al*., 1993; Komolong *et al*., 2003).

Ergometrine drug has long been used for strong uterine contraction and control of postpartum haemorrhage due to its pronounced effect on direct stimulation of the rate and force of rhythmical contractions and ability to cause smooth muscle tissue of blood vessel walls to narrow, thereby reducing blood flow (Daniel and Maria, 2000; Dele - Ojeme, 2002). This drug is produced from ergot alkaloids formed during the infection process of a perennial grass -*Serale cereale* by a smut fungus - *Claviceps purpurea*. (Hudler, 1998; Tudzynski *et al*., 2001). The Rye plant being a temperate plant may not grow in the tropical climate thereby making commercial production of the ergot alkaloids in Nigeria (tropics) difficult. Therefore, huge amount of money is spent annually in importing this drug from western countries.

Panicum maximum is a perennial tufted grass indigenous to Africa and widely distributed throughout the tropics and subtropics (Arohkesi, 1997; Aganga and Tshwenyane, 2004). In addition to its being classified among the best forage grass due to its high nutritive value, its roles in phytoremediation and control of soil erosion has been reported by several workers (Mellory, 1972; Olabode *et al*., 2001; Merki *et al*., 2004; Ogbo *et al*., 2009).

Panicum maximum, in a similar way to temperate rye plant has been found to be susceptible to the smut disease. The causative agent in this case has been identified to be Tilletia ayresii (Kanife, 2011). However; there is a dearth of information on the contractility properties of the secondary metabolites that may result from the disease infection. This study was therefore designed to investigate the uterine contractility potentials of the solvent extracts of the bioactive components of infected florets of *Panicum maximum* (tropical plant) in comparison with the orthodox (standard) ergometrine. Although antidiabetic and antibacterial property of ethanolic leaf extract of *P. maximum* plant have been reported, there is no report on its contractility-property as well as the bioactive component responsible for the contractility of infected *P. maximum* florets.

MATERIALS AND METHODS

Plant material and preparation of extracts

Two kilograms of infected *Panicum maximum* florets were harvested from specially cultivated beds within the premises of University of Lagos, Akoka, Nigeria between the months of June- November 2010. The

samples were identified and authenticated by taxonomist and Mycologist (Profs. Olowokudejo and Adekunle) of Department of Botany. The voucher specimen (LUTH 3687) was kept in the University Herbarium. The sample was washed, dried, pulverized and kept in sterile chamber until use. The powdered sample was subjected to sequential extraction procedure according to the method of Sofowora (1982) using the following solvents: Distilled water (4 litres), analytical grade of chloroform (6litres) and ethanol (5 litres). The extracts were concentrated in the rotatory evaporator under reduced pressure and controlled temperature (40^{o}C). The dried solid products were weighed to determine yield and transferred to airtight containers. They were stored in the refrigerator until used. The % yield was calculated using the expression:

$$\% \text{ Yield} = \frac{\text{Weight of dry extract} \times 100}{\text{Weight before extraction 1}}$$

For bioactivity investigation, the extracts (aqueous, chloroform and ethanol) were dissolved in 5% Tween 80 in order to prepare different final organ bath concentrations.

Fractionation of ethanol extract was done by Column Chromatograph according to the methods of Dufresne and Salituro (1998). Forty grams of ethanol extract was subjected to Column Chromatography with silica gel 60-200 mesh as stationary phase. The mobile phases were N-hexane, ethylacetate, ethanol and methanol. The four fractions obtained were concentrated with rotatory evaporator at very low temperature and pressure. The dried powdery samples were stored in the refrigerator until used. Of the four fractions, the most active (ethanol fraction) after bioassay was further purified with Thin layer Chromatography (TLC) which with detection under ultraviolet (UV) light ($F_{256\ -\ 366nm}$). The R_f value of each compound detected was calculated as:

$$\frac{\text{Distance moved by solute}}{\text{Distance moved by solvent}}$$

The resulting compounds isolated were subjected to bioassay to determine the most active compound.

Preparation of experimental animals

Thirty adult female *Sprague-dawley* rats weighing between 140-180g procured from animal house of the College of Medicine of the University of Lagos were used. They were kept in standard metal cages and had access to standard rat chow (Nimeth livestock feeds, Ikeja, Nigeria) and water *ad libitum*. The animals were acclimatized for 7 days at temperatures of 28 ± 31^{o}C with 12hr: 12hr light: darkness periodicity before bioassay.

Assessment of uterine contractility

The rats were pretreated with 1.5mg/kg body of stilboesterol given orally 24 hours before the experiment to ensure regular spontaneous uterine contraction. The rats were sarcrified by cervical dislocation and the uterus excised. The uterine horns were placed in cold DE- Jalon's solution in order to reduce enzymatic activity and then trimmed of excess connective tissue. The uterine horns were carefully cut into 2.0-3.0mm ring segments and mounted in 20ml organ baths containing De-Jalon's solution consisting of NaCl = 9g, $NaHCO_3$ = 0.5g, Glucose = 0.5g, 10%KCl = 4.2ml, 1M $CaCl_2$ = 0.27 in 1 litre. The organ bath was bubbled with 95% O_2 - 5% CO_2 gas mixture. The temperature and pH were maintained at 37^{o}C and 7.4 ± 0.2, respectively. The rings were connected to a force transducer (Grass Model TO_3), which was coupled to a 4-Channel Grass Model 7D polygraph for the recording of the isometric tension. Each tissue was allowed to equilibrate for 60 - 90 minutes under resting tension of 1g according to the methods of Calixto *et al.* (1991) and Sofola *et al*., (2008). At the end of the equilibration period cumulative aliquots of the infected aqueous, ethanol and chloroform extracts were added to the organ bath in the absence of any agonist. The contractile responses of the uterine segment to the aliquots were recorded. This experiment was repeated using graded concentration of Ergometrine maleate for equilibration (10^{-5} - 10^{-2} mol/L) and their effects noted. The final bath concentrations of the infected extracts in mg/ml were 0.0312, 0.625, 0.125, 0.25, 0.5, 1.0 and 2.0, respectively. Bioassay of the four fractions was also carried out on uterine muscle stimulated by ergometrne and unstimulated muscles and percentage responses calculated.

Statistical analysis

Data are expressed in mean ± SEM. One-way analysis of variance (ANOVA) was carried out in all experiments. The contractile responses were expressed as percentage of the maximal response. The computer software used for the analysis is SPSS 11.0. A p-value of less than 0.05 was considered significant.

RESULTS

Sequential extraction of infected *Panicum maximum* florets (PMF) yielded varying percentage weight of crude extracts in different solvents used (aqueous = 1%, chloroform = 2.6%, ethanol = 5%). Ergometrine (orthodox drug) is known to cause contraction of uterine muscles and in this experiment it produced a dose-dependent contraction of non-pregnant uterine muscle (Figure-1). The aqueous, ethanol and chloroform extracts of infected (Pmf) produced a dose-dependent contraction of the primed non-pregnant uterine muscle (Figure-2). Ethanol extract produced the highest contraction with the least dose achieving 77% contraction at final bath concentration of 0.062mg/ml. The infected ethanol extract fraction produced a similar effect on ergometrine- stimulated uterine muscles with greater contraction compared with the unstimulated uterine muscles. However, the patterns of contraction of the two are similar (Figure-3). Further fractionation of the infected ethanol extract (most active) and elution with four solvents yielded four fractions. Out of the four fractions produced, only ethanol fraction was active while others were inactive. Further separation of the active ethanol fraction

on TLC plate revealed the presence of three compounds with the R_f values-of 0.20, 0.30 and 0.90, respectively. Contractility studies of the three compounds showed that only the compounds with R_f value = 0.20 was active while the other compounds were inactive.

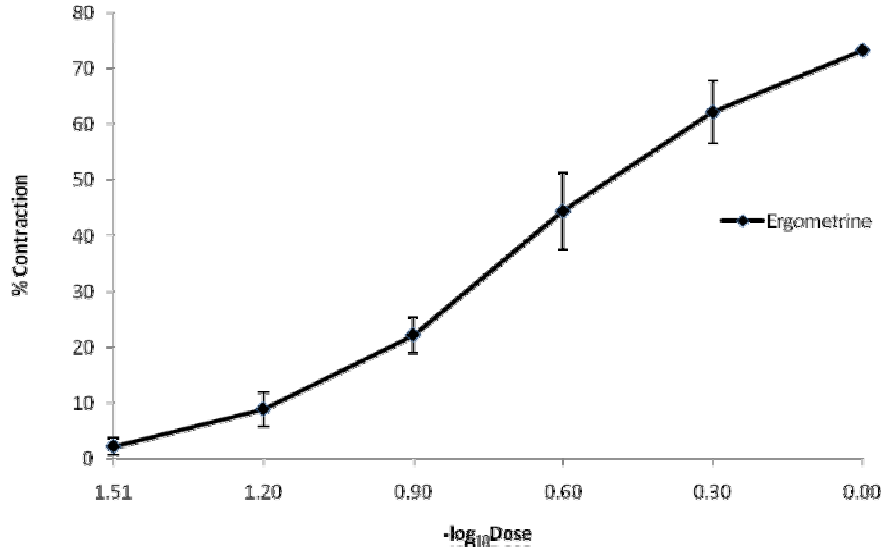

Figure-1. Effect of graded concentration of ergometrine on uterine smooth muscle of non-pregnant rat. Response is presented as percentage contraction.

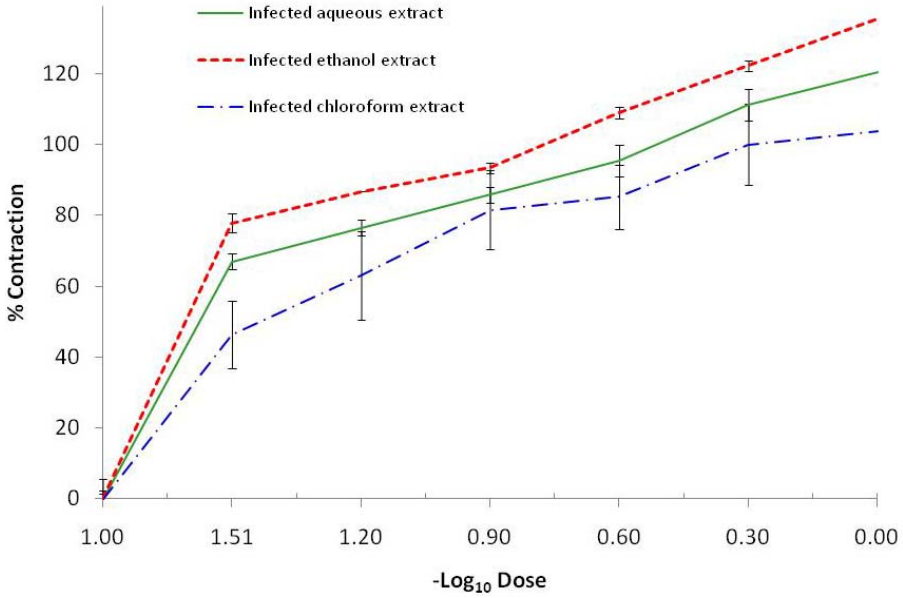

Figure-2. Effect of graded concentrations of aqueous, ethanol and chloroform extracts of infected *P. maximum* florets on uterine smooth muscle. Response is presented as percentage contraction.

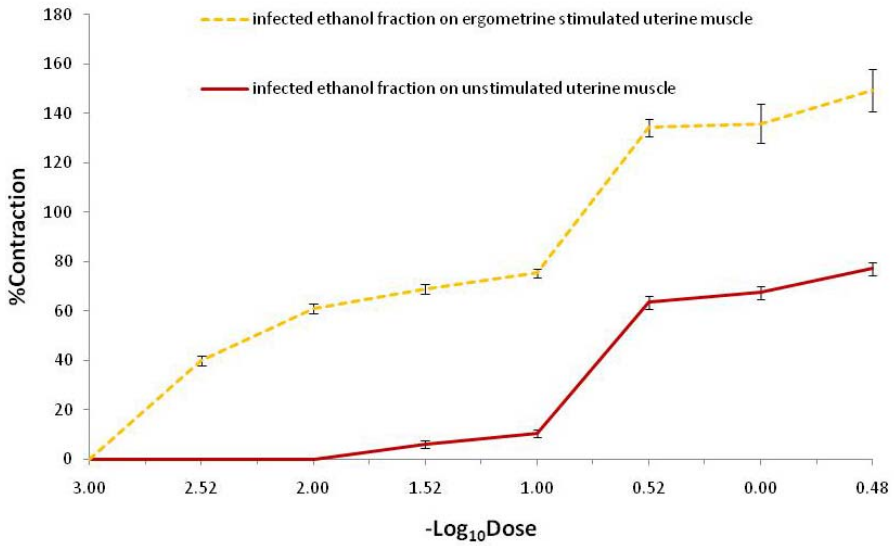

Figure-3. Effect of graded concentrations of active ethanol fraction of infected *P. maximum* florets on stimulated and unstimulated uterine smooth muscles. Response is presented as percentage contraction.

DISCUSSIONS

Clinically, drugs used to induce labour contract uterine muscles. Some of these drugs are oxytocin and ergometrine. The use of ergometrine in obstetrics for induction of labour to facilitate delivery of placenta and prevent bleeding after childbirth through vasoconstriction has been reported (Vandongen and Groot, 1995; Hudler, 1998; Boicheuko *et al*., 2001; Lee, 2009). It is known that contraction induced by agonists is mainly due to calcium influx through the voltage-gated calcium channels opened directly or indirectly by agonist receptor (Ruttner *et al*., 2000; Ruttner *et al*., 2002). In the present study, different doses of aqueous, ethanol and chloroform extracts of infected *P. maximum* florets produced progressive increase in uterine contraction suggesting that the extracts did not inhibit calcium influx through the voltage-gated calcium channels.

The extract despite being crude exhibited good potential as an oxytocic agent. Small doses of the extracts increased amplitude of spontaneous uterine contraction while large doses sustained it. The onset of action exhibited by the extracts could merely reflect the high concentration of active compound present. However, ethanol extracts showed higher contraction than the aqueous and chloroform. This could be due to its ability to extract more active component than others. This result suggests the presence of ergometrine-like substances in the crude infected *Panicum maximum* extract.

Furthermore, a production of a similar pattern of contraction on uterine muscles when ethanol fraction was tested on unstimulated and ergometrine-stimulated uterine muscles is an indication that the fraction contains an active component similar to that of ergometrine. The presence of three spots detected on TLC plate suggests presence of three components in the pure fraction. The active compound with Rf value of 0.20 identified to be an alkaloid is been further characterized for possible formulation into drug for uterine contraction.

CONCLUSIONS

This crude and partially purified ethanolic extracts of infected *Panicum maximum* floret study (tropical plant) exhibited contractility effect similar to ergometrine normally used to induce uterine contraction and subsequent control of postpartum haemorrhage in the tropics and so could serve as an alternative to ergometrine.

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