Effects of *Sesamum Radiatum* Aqueous Leaf Extract on Rhythmic Contractions of Uterine Smooth Muscle Bundles from Pregnant Rats

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Abstract- ***Sesamum radiatum*** **is employed in traditional medicine for parturient women to facilitate deliveries. In this study, the effects of the aqueous leaf extract were examined on the contractile activity of uterine smooth muscle isolated from pregnant Wistar rats (19-21 days). The isometric contractile force of the uterine smooth muscle bundles was recorded by using a strain gauge.** ***S. radiatum*** **aqueous leaf extracts (ESera, 1×10^{-4} µg/ml - 100 µg/ml) showed uterotonic properties. These uterotonic effects were characterized by the increase of the amplitude, the frequency and the basal tone of the uterine smooth muscle strips in normal Mac Ewen solution and by the development of contracture in depolarizing solution and in solution without calcium. Similar effects were observed with Oxytocin (OT, 2.5×10^{-14} µg/ml - 2.5×10^{-9} µg/ml) and misoprostol (Miso, 1×10^{-3} µg/ml – 0.08 µg/ml). In Ca^{2+}-free solution, the addition of the ESera (10 µg / ml) elicited the development of contracture in the presence of EGTA (0.1 mM). This result suggests that ESera could act on the double calcium flux (intracellular and extracellular) like misoprostol, a synthetic analogue of prostaglandin E_1. In conclusion, the aqueous leaf extract of** ***S. radiatum*** **(ESera) had uterotonic effects (prostaglandin-like activity) on uterine contraction in pregnant rats.**

Keywords- Sesamum Radiatum; Oxytocin; Misoprostol; Uterus; Contractile Activity

I INTRODUCTION

In developing countries, low economic resources limit the capacity of people to buy pharmaceuticals. One consequence of this is desertion or late visits to health facilities [1]. In these countries, less than 20% of women have access to institutional delivery services. For them, home birth is not a choice, it is almost inevitable. Obviously, they use medicinal plants to facilitate delivery [2]. *Sesamum radiatum* Schum. & Thonn. (Pedaliaceae) is one of the plants used in this field. In Côte d'Ivoire, this plant species grows on the whole territory, on the lands and even around habitations. The leaves are often used as oxytocic-like for parturient to facilitate deliveries [3-5].

Previous works showed that the effects of *S. radiatum* aqueous leaf extract (ESera) on blood pressure could be an indicator of its use in traditional medicine to facilitate deliveries in parturient women [6, 7]. ESera induced a dose-dependent hypotension resulting from cardiodepression and vasorelaxation [6]. The vascular relaxation was endothelium-dependent and mainly involved nitric oxide (NO) pathway [7]. The effects of ESera on blood pressure were comparable to those observed with *Caesalpinia bonduc* and *Mareya micrantha*, two uterotonic plants used traditionally to facilitate childbirth [8, 9]. Similar effects were observed with pharmacodynamic substances (oxytocin and prostaglandin) employed in obstetrical practice to stimulate labour. The hypotension induced by these substances could contribute to parturition. Indeed, the pharmacological modification during pregnancy can be source of a possible hemorrhage [10]. According to [11], oxytocic-like substances only induce a hypotensive effect which can minimize the heavy bleeding during the delivery.

So it is clear that these pharmacodynamic substances are used in obstetric practice for two reasons: reduction of blood pressure and uterotonic property. Indeed, uterine contraction plays a fundamental role in labour. According to some authors, labour failure is partly due to dysfunction of uterine contraction argued that Ca^{2+} mobilization is essential during uterine contraction even if the mechanisms underlying this activity of uterus are not completely understood [12-17].

The aim of the present study was to examine the direct effects of *S. radiatum* aqueous leaf extract (ESera) on rat uterine contractility. Thus, in this paper, data investigating the following aspects were presented: (1) the effects on spontaneous contractions, (2) the effects on Ca^{2+} mobilization and depolarizing action and (3) a comparison with those of two pharmacodynamic substances (Oxytocin and misoprostol) known and commonly used in obstetrical practice to stimulate labour [18].

II MATERIALS AND METHODS

A. Ethical Considerations

Experimental procedures and protocols used in this study were approved by Ethics Committee of Félix Houphouët-Boigny University. These guidelines were in accordance with the internationally accepted principles for laboratory use and care [21].

B. Plant Material

1) Collect of Plant:

Fresh leaves of *S. radiatum* were collected in October 2005 in a forest of the Southern region of Côte d'Ivoire (Region des Lagunes). The leaves of *S. radiatum* were certified to be an identical sample at the specimen herbarium of National Plant Centre (Centre National Floristique) of Côte d'Ivoire at Cocody University in Abidjan. Voucher specimen were preserved and catalogued in the same herbarium (Voucher specimen n° 8948, *Sesamum radiatum* L. of 17 June 1966 and *Sesamum radiatum* voucher n° 11616 of June 1974 in Dabou). This pantropical plant was authenticated by a Botany expert, Prof. Ake-Assi Laurent of National Plant Centre, UFR-Biosciences, Félix Houphouët-Boigny University, Abidjan, Côte d'Ivoire.

2) Extract Preparation:

The methods were previously described [22].

C. Animals and Tissue Preparation

Pregnant Wistar rats (19-21 days after conception), weighing between 250 and 300 g were used. The animals were kept and maintained under conventional laboratory conditions of temperature, humidity and light/dark cycle of 14/10 hours and allowed free access to food (standard pallet diet) and drinking tap water *ad libitum*. All animals were fasted for hours, but still allowed free access to drinking water, before the commencement of experiments.

The rats were sacrificed by cervical dislocation and were quickly placed on a dissecting table. After a median laparotomy, uterine horns were isolated, removed and transferred to normal saline solution (Mac Ewen). The two uterine horn segments were cleaned free fat and connective tissues and cut into longitudinal strips (5-6 mm long, 1-2 mm wide). Dissection was performed under binocular microscope (Leitz-Wild, Germany). The Pregnant Rat Uterus Preparations (PRUPs, uterine smooth muscle bundles) were transferred in a Petri dish containing a normal Mac Ewen solution with following composition (mM): NaCl, 130; KCl, 2.5; $CaCl_2$, 2.4; NaH_2PO_4, 1.18; $NaHCO_3$, 11.9; $MgCl_2$, 0.24; glucose, 2.2). The solution (pH of 7.4) was kept at a temperature of 35 ℃ [23].

D. Measurement of Isometric Contractile Force

1) Tissue Preparation Mounting:

The methods were previously described [22]. The both chambers of the tissue baths contained the appropriate Mac Ewen solution (37 ℃) [23, 25] constantly bubbled with carbogen (95% O_2, 5% CO_2), giving a pH of 7.4. Isometric force was measured and recorded using a Multipen Recorder Rikadenki polygraph (HSE, Freiburg, Germany) at a speed of 2.5 mm/min. A pre-load of 1 g was applied. Changes in isometric force were measured and recorded by means of a force transducer.

2) Experimental Protocol:

After mounting, preparations were allowed to equilibrate until the spontaneous phasic contractions became stable (60 min). The tissue was then challenged with 60 mM KCl to ensure contractile viability and determine maximum contraction. After equilibration, contractions were stimulated by bath exposure of bundles to *S. radiatum* extract (1×10^{-4} µg/ml - 100 µg/ml), misoprostol (1×10^{-3} µg/ml – 0.08 µg/ml) and oxytocin (2.5×10^{-14} µg/ml - 2.5×10^{-9} µg/ml) separately. These substances were added to the organ bath cumulatively [26]. To assess whether the spasmogenic activities of the test substances were through calcium channel, the tissue (PRUPs) was allowed to stabilize in normal Mac Ewen solution, which was then replaced with Ca^{2+}-free solution containing EGTA (0.1 mM) for 12 min to remove Ca^{2+} from the tissue. The Ca^{2+}-free solution was obtained with the salts used to prepare normal Mac Ewen solution but without $CaCl_2$. To confirm the depolarizing action of substances, High-K^+- and Ca^{2+}-free solutions containing EGTA (0.1 mM) were used to depolarize the preparations [27]. High-K^+ solution was obtained by substituting 70 mM NaCl with KCl.

E. Chemicals Used

The following reference drugs were used: Oxytocin (Syntocinon®, Novartis, France), misoprostol (Cytotec®, Pharmacia Limited, United Kingdom) and ethyleneglycol-bis(aminoethyl ether) *N,N,N',N'*-tetraacetic acid (EGTA, Sigma Chemical Company, USA). All drugs were dissolved and/or diluted in distilled water on each day of our experiments [28]. Drugs concentrations quoted in the text refer to final organ-bath concentration.

F. Data Analysis

Data obtained from η separate experiments were expressed as means (± standard errors of the means, SEM). Statistical analysis and graphics were carried out using the software GraphPad Instat and GraphPad Prism 4 (San Diego, California, USA), respectively. Statistical analysis of the results was determined by using the unpaired Student's *t*-test. $p < 0.05$ was considered as indicative of significance.

III RESULTS

A. Dose-Dependent Effects of Studied Substances on Rhythmic Contractions

1) Effects of S. Radiatum Aqueous Leaf Extract:

S. radiatum aqueous leaf extract (ESera) was tested on PRUPs with increasing concentrations ranging from 1×10^{-4} µg/ml to 100 µg/ml. ESera increased the contractile activity of PRUPs (Table 1). The force and the rate of the contractions increased. In the absence of ESera, the contractile force was estimated at 321 ± 23 mg. It reached 400 ± 31.5 mg ($p > 0.05$) and 1275 ± 33.8 mg ($p < 0.001$) when ESera was used respectively at 1×10^{-4} µg/ml and 100 µg/ml. Those augmentations of contractile force

TABLE I DOSE-DEPENDENT EFFECTS OF *S. RADIATUM* AQUEOUS LEAF EXTRACT ON THE CONTRACTILE ACTIVITY OF UTERINE SMOOTH MUSCLE ISOLATED FROM PREGNANT RAT

ESera (µg/ml)	Contractile Force (mg)	Increase of CF (%)	Increase of RC (%)
0 (Control)	321 ±23	-	-
1×10^{-4}	400 ±31.5^{ns}	24.6 ±3.9^{ns}	-
1×10^{-3}	500 ±19.5**	55.8 ±7.2^{ns}	-
0.01	540 ±24.9***	68.2 ±11.1^{ns}	-
0.1	585 ±17.1***	82.2 ±8.4*	5 ±1.4^{ns}
1	762 ±38.6***	137.4 ±9.2***	10 ±2.8^{ns}
10	937.5 ±30.8***	192.1 ±14.3***	30 ±6***
100	1275 ±33.8***	297.2 ±12.5***	45 ±6.5***

S. radiatum aqueous leaf extract (ESera) applied in a range of concentrations from 1×10^{-4} µg/ml to 100 µg/ml caused the increase of the contractile activity of the isolated uterine smooth muscle. The contractile force (CF) and the rate of contractions (RC) were increased in concentration-dependent manner. Data shown are mean ±S.E.M. (n = 6). *ns*, $p > 0.05$ vs. control; *, $p < 0.05$ vs. control; **, $p < 0.01$ vs. control; ***, $p < 0.001$ vs. control.

corresponded to respective increases of 24.6 ± 3.9 % ($p > 0.05$) and 297.2 ± 12.5 % ($p < 0.001$) compared to the control. The rate of contractions was only affected by high concentrations. Indeed, the low concentrations of ESera (1×10^{-4} µg/ml to 0.01 µg/ml) did not alter the rate of contractions of PRUPs. But beyond 0.01 µg/ml, an increase of the frequency of the contractions was observed. Thus, at the concentration of 0.1 µg/ml, the frequency was increased from 5 ± 1.4 % ($p > 0.05$) and attained 45 ± 6.5 % ($p < 0.001$) for the concentration of 100 µg/ml. This effect of ESera on the PRUPs was concentration-dependent with EC_{50} value (95% confidence limits) of 8.3 µg/ml.

2) Effects of Oxytocin:

The addition of increasing concentrations of oxytocin (OT) into the organ bath caused a concentration-dependent increase of the contractile activity of PRUPs (Table 2). That uterotonic action of OT was characterized by the elevations of the contractile force and the rate of contractions. The initial contractile force recorded was 504 ± 27 mg. When OT was used at 1×10^{-5} µIU/ml, the measured contractile force was 588 ± 22 mg ($p > 0.05$) giving an increase of 16.7 ± 2.5 % ($p < 0.05$). This increase of the amplitude of the contractions was progressive and reached the value of 1428 ± 18 mg ($p < 0.001$) with the high concentration of 1 µIU/ml corresponding to an increase of 183.2 ±17.3% ($p < 0.001$). The calculated EC_{50} (95% confidence limits) was 7×10^{-3} µIU/ml. The rate of the contractions remained unaltered when low concentrations (1×10^{-5} µIU/ml to 1×10^{-4} µIU/ml) were applied. For high concentrations ranging from 1×10^{-3} µIU/ml to 1 µIU/ml, an elevation of the rate of contractions was noticed. OT at 1×10^{-3} µIU/ml elicited increases of 12 ± 2.5 % ($p > 0.05$) for the rate of contractions. This increase was more important at the high concentration of 1 µIU/ml. At this concentration, the rate of contractions was increased from 43.8 ± 6.5 % ($p < 0.001$).

TABLE II DOSE-RESPONSE EFFECTS OF OXYTOCIN ON THE CONTRACTILE ACTIVITY OF THE UTERINE SMOOTH MUSCLE ISOLATED FROM PREGNANT RAT

Oxytocin (µIU/ml)	Contractile Force (mg)	Increase of CF (%)	Increase of RC (%)
0 (Control)	504 ±27	-	-
1×10^{-5}	588 ±22^{ns}	16.7 ±4.8^{ns}	-
1×10^{-4}	764 ±28.9***	51.6 ±8*	-
1×10^{-3}	1044 ±31.5***	107.1 ±9.9***	12 ±2.5^{ns}
0.01	1248 ±40.8***	147.6 ±7.6***	25 ±4.2***
0.1	1308 ±41.1***	159.5 ±12.5***	37 ±7***
1	1428 ±32.5***	183.3 ±17.3***	43.8 ±6.5***

Oxytocin, employed in a range of concentrations from 1×10^{-5} µIU/ml to 0.1 µg/ml, induced a progressive increase of the contractile force (CF) and the rate of contraction (RC) of the uterine smooth muscle bundles of pregnant rat. Data shown are mean ±S.E.M. (n = 6). *ns*, $p > 0.05$ vs. control; *, $p < 0.05$ vs. control; **, $p < 0.01$ vs. control; ***, $p < 0.001$ vs. control.

3) Effects of Misoprostol:

Misoprostol (Miso), analogue of prostaglandin E_1 (PGE_1) was applied to the strips of uterus (PRUPs). The results obtained are shown in Table 3. Miso (1×10^{-3} µg/ml - 0.08 µg/ml) concentration-dependently induced an increase in contractile activity of PRUPs with EC_{50} value (95% confidence limits) of 0.03 µg/ml. The initial contractile force (Control) of PRUPs was 668.2 ± 26.3 mg. When Miso was administered at 1×10^{-3} µg/ml, contractile force increased to 837 ± 16.1 mg ($p > 0.05$). This augmentation of the contractile force represented an increase of 25.2 ± 1.3 % ($p > 0.05$). A high concentration of Miso (0.08 µg/ml) triggered a significant contractile force of 1839.3 ± 21.5 mg ($p < 0.001$), corresponding to an increase of 175 ± 14.8% ($p < 0.001$). This increase in contractile force was accompanied by that of the frequency of contraction. The low concentrations of Miso (1×10^{-3} µg/ml to 0.01 µg/ml) did not change the frequency of contractions. However, when high concentrations were used, an elevation of the basal tone was recorded. The rate of contractions was evaluated to 10.7 ± 2.8 % ($p < 0.05$) and 57 ± 7.2 % ($p < 0.001$) respectively following the application of Miso at 0.02 µg/ml and at 0.08 µg/ml.

TABLE III DOSE-DEPENDENT EFFECTS OF MISOPROSTOL ON THE CONTRACTILE ACTIVITY OF THE UTERINE SMOOTH MUSCLE ISOLATED FROM PREGNANT RAT

Misoprostol (µg/ml)	Contractile Force (mg)	Increase of CF (%)	Increase of RC (%)
0 (Control)	668.2 ± 26.3	-	-
1×10^{-3}	837.5 ± 16.1^{ns}	25.3 ± 4^{ns}	-
0.01	1237.5 ± 22.2***	85.2 ± 7.2^{ns}	-
0.02	1375 ± 26.5***	105.8 ± 9.1^{ns}	10.7 ± 2.8^{ns}
0.04	1475 ± 37.1***	120.7 ± 12.1*	21.4 ± 3.5*
0.06	1706.3 ± 75.8***	155.4 ± 9.9***	35.7 ± 6.8***
0.08	1839.3 ± 56***	175 ± 14.8***	57 ± 7.2***

Misoprostol (1×10^{-3} µg/ml to 0.08 µg/ml)) elicited a progressive increase of contractile activity of the uterine smooth muscles. The two parameters studied, the contractile force (CF) and the rate of contraction (RC) are augmented. Data shown are mean ± S.E.M. (n = 6). *ns*, $p > 0.05$ vs. control; *, $p < 0.05$ vs. control; **, $p < 0.01$ vs. control; ***, $p < 0.001$ vs. control.

B. Comparative Effects of Studied Substances on Ca^{2+}-Mobilization and Depolarizing Actions

1) In Ca^{2+}-Free Mac Ewen Solution:

Removing extracellular calcium abolished spontaneous contractions, whereas the basal tone was maintained. When ESera, OT and Miso were introduced separately in the isolated organ bath, an increase in the basal tone of PRUPs was recorded. It was contracture (Fig. 1A). This contracture reached values of 56.25 ± 3.6 mg ($p < 0.001$), 25 ± 2 mg ($p < 0.01$) and 62.5 ± 4 mg ($p < 0.001$), respectively, after exposure to ESera (10 µg/ml), OT (7×10^{-3} µIU/ml) and Miso (0.03 µg/ml). The pre-treatment of the tissues with EGTA (0.1 mM) did not abolish the contractures induced by ESera and Miso. The maximum values obtained were 43.8 ± 2.1 mg ($p < 0.001$) for ESera and 47.5 ± 3.5 mg ($p < 0.01$) for Miso. However, the presence of this chelator of calcium in the solution strongly affected the effect of OT. No significant change in basal tone was recorded. Indeed, in this solution containing EGTA, OT caused a slight increase of basal tone evaluated to 7 ± 2 mg ($p < 0.05$) (Fig. 1B).

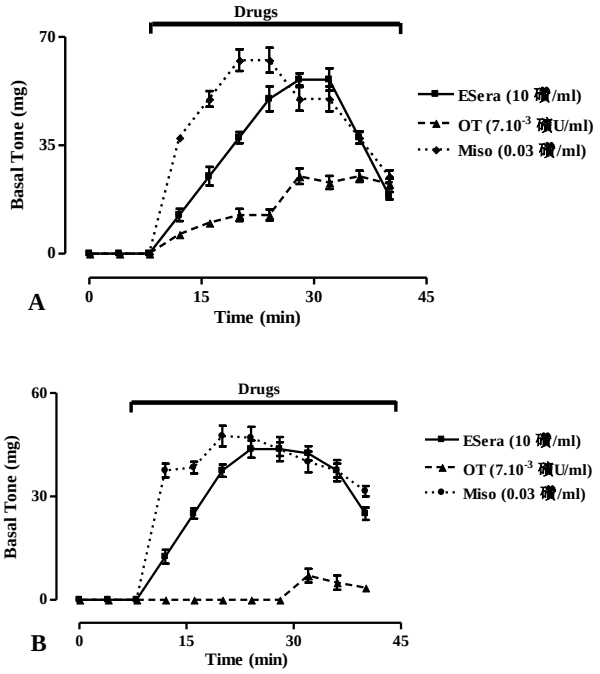

Fig. 1 Effect of the studied substances on the contractile activity of the uterine smooth muscle bundles placed in the calcium-free solution.
Employed alone, the studied substances induced an increase of the basal tone of the uterine smooth muscle bundles isolated from pregnant rat (J19-J21). It is a contracture (A). The addition of EGTA (0.1 mM) in this solution did not suppress the contracture developed by ESera (10 µg/ml) and Miso (0.03 µg/ml). However, oxytocin-induced contracture (OT,

7×10^{-3} µIU/ml) was significantly affected by the presence of the EGTA (B). Data shown are mean ± S.E.M. (n = 5-6; $p < 0.05$).

2) In High-K^+ - and Ca^{2+}-Free Mac Ewen Solution:

The results recorded in this solution were similar to those obtained in the solution above (Fig. 2). ESera (10 µg/ml), Miso (0.03 µg/ml) and OT (7×10^{-3} µIU/ml) showed an increase in basal tone of PRUPs (Fig. 2A) which persisted in the presence of EGTA (0.1 mM) for ESera and Miso (Fig. 2B). Indeed, the administration of ESera in these conditions (70 mM K^+ - $0Ca^{2+}$) was found to produce a contracture of 19 ± 1.6 mg ($p < 0.001$) and when EGTA was added to the same medium, a contracture of 18.75 ± 2 mg ($p < 0.001$) was determined. With Miso, contracture values of 43.8 ± 3 mg ($p < 0.001$) and 37.5 ± 3.5 mg ($p < 0.001$) were assessed respectively in the absence and the presence of EGTA. The use of EGTA significantly reduced the increase of basal tone induced by OT (7×10^{-3} µIU / ml). Thus, in the absence of EGTA, a contracture of 18.8 ± 2.5 mg ($p < 0.001$) was measured whereas PRUPs pre-treated with EGTA induced a contracture of 5 ± 1.3 mg ($p > 0.05$).

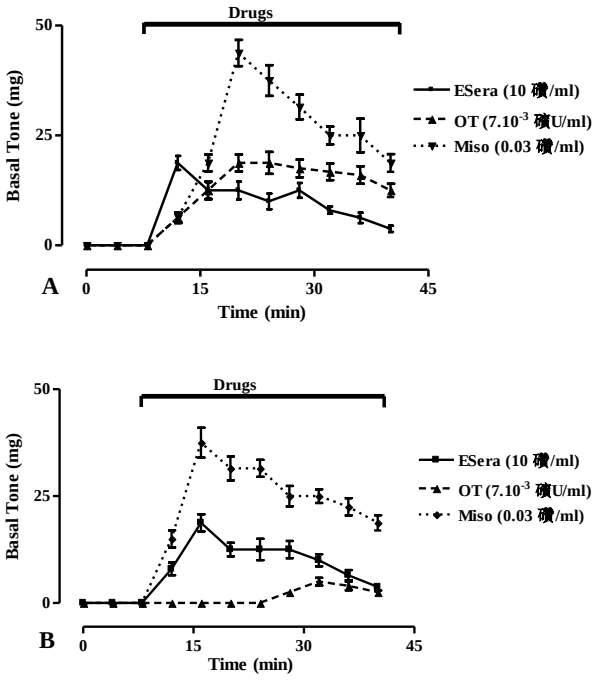

Fig. 2 Effects of the test substances on the contractile activity of the uterine smooth muscle bundles in high-K^+ - and Ca^{2+}-free solution.
In the absence of EGTA (0.1 mM), the test substances caused the contracture of the uterine smooth muscle bundles of pregnant rat (A). Contractures induced by ESera (10 µg/ml) and Miso (0.03 µg/ml) even persisted whereas that of OT (7×10^{-3} µIU/ml) was significantly affected in presence of the EGTA (B). Data shown are mean ± S.E.M. (n = 5-6; $p < 0.05$).

IV DISCUSSION

The aqueous leaf extract of *Sesamum radiatum* (ESera) caused an increase in contractile activity of uterine muscle. The amplitude and frequency of spontaneous contractions were significantly increased. These effects of ESera were similar to those of *Parquetina nigrescens* [29], *Caesalpinia bonduc* [23], *Mareya micrantha* [30], *Plumbago rosea* [31] and *Ficus exasperata* [32], which are medicinal plants often used to facilitate childbirth.

These results were also similar to those of oxytocin and misoprostol (a synthetic analogue of PGE_1). The effects of oxytocin were described [12]. The effects of the misoprostol were investigated [33]. Oxytocin and misoprostol, because of their uterotonic property, are used in gynecology and obstetrics to induce and/or facilitate labour [18, 34, 35].

In the Ca^{2+}-free solution, ESera elicited contracture of uterine smooth muscle. This observation suggested that calcium influx in these conditions could not be responsible for the contracture. Therefore, it was probable that calcium was provided by another source.

To elucidate this hypothesis, the effects of ESera were investigated on fragments of uterus immersed in Ca^{2+}-free solution and supplemented with EGTA, a specific chelator of calcium ion (Ca^{2+}). The aqueous leaf extract of *S. radiatum* (ESera) also produced a contracture of the uterine muscle in this solution. In other words, the contracture was maintained in the presence of EGTA. Similar results were observed with misoprostol and sulprostone, a synthetic analogue of PGE_2 [23, 29, 36]. The contracture induced by ESera and misoprostol could therefore be strongly due to the mobilization of intracellular calcium. Such effects were recorded with several other urotonic plant extracts such as *Parquetina nigrescens* [29], *Caesalpinia bonduc* [23] and *Carica papaya* [37].

With oxytocin, the presence of EGTA significantly affected the contracture recorded in the Ca^{2+}-free medium. The results obtained with oxytocin corroborated those of previous studies achieved by many other authors.

Indeed, according to [38], removing extracellular calcium abolished spontaneous contractions of uterine smooth muscle. Subsequent addition of oxytocin caused transient increases in contraction in a concentration-dependent manner. These increases were significantly smaller than those seen in the presence of extracellular calcium. References [15, 39] also showed that removal of external Ca^{2+} caused a significant decrease in the oxytocin-induced rise in calcium and force production.

The requirement for calcium in uterine contractions attracted attention as early as 1909 [40]. During subsequent years, great progress was made in understanding the mechanisms of action of calcium leading to this contraction. It is now well established that a rise in intracellular calcium in uterine smooth muscle causes activation of myosin light chain kinase and phosphorylation of myosin light, leading to contraction [39]. The rise in calcium, as in many different cell types, is traditionally believed to be from two sources: extracellular calcium entry through plasma membrane calcium channels and release of calcium from the sarcoplasmic reticulum. The intracellular calcium plays a key role in the

development of the slow component of the contractile activity, the basal tone of uterine smooth muscles [41, 42]. It was demonstrated that removal of external Ca^{2+} prevents the rise of Ca^{2+} needed for spontaneous contraction. The basic phasic nature of uterine contractions, which is essential for successful labour, is critically dependent on Ca^{2+} influx through voltage-gate L-type Ca^{2+} channels [15].

Agonists like oxytocin and prostaglandins can increase the force and the frequency of rhythmic contractions [43], which are also suppressed by L-type Ca^{2+} channel blockers or by removal of extracellular Ca^{2+} [44]. Therefore, Ca^{2+} influx through L-type Ca^{2+} channels is important in Ca^{2+} regulation in both spontaneous and agonist-stimulated rhythmic contraction of uterine smooth muscle cells [38]. These substances can increase $[Ca^{2+}]_i$ both by enhancing Ca^{2+} influx through L-type Ca^{2+} channels and by causing IP3-induced Ca^{2+} release from the intracellular Ca^{2+} stores [15].

The effects of ESera on the uterus were also evaluated in high potassium Ca^{2+}-free (depolarizing solution) and EGTA-supplemented high potassium Ca^{2+}-free media. Like misoprostol, the aqueous leaf extract of *S. radiatum* (ESera) caused contracture of the uterus isolated from pregnant rats in the hyperpotassic solutions. This contracture which was maintained due to the effect of ESera, showed that this extract was a stimulating and depolarizing agent. It is likely to mobilize intracellular calcium and thus raise the slow calcium component, namely the tonic contraction of the uterus. It may also act on the rapid component of calcium (calcium influx), which according to [45] depends on membrane potential. Indeed, the introduction of the uterine fragments in the hyperpotassic solution caused a relaxation of the muscle. The addition of calcium (1 mM) in the same medium led to a sustained contracture. The same effects were observed [23]. According to [45], this rapid relaxation in the high K^+ medium was a rapid phase of the contractile response, followed by a release of Ca^{2+} from internal calcium stores. The relaxation of the myometrium is related to the existence of ATP-dependent calcium pumps on the reticulum membranes. In these experimental conditions, relaxation would be attributed to a depletion of calcium in the myometrium [46]. Contracture observed in the solution by addition of calcium, clearly indicated that calcium was the main activating factor [47]. Subsequently, it was a calcium influx that triggered a release of free ionized calcium from the internal stores.

Reference [48] showed that when the external potassium concentration was increased to 40 mM, in Ca^{2+}-free solution, sodium-calcium interaction led to a predominance of calcium component therefore explaining the development of contracture.

V CONCLUSION

In conclusion, the aqueous leaf extract of *S. radiatum* (ESera), misoprostol and oxytocin produced a stimulating effect on uterine smooth muscle contraction. ESera had a uterotonic action. This uterotonic effect was characterized by an increase in the rate and amplitude of contractions in normal Mac Ewen solution and the development of contracture in depolarizing medium (calcium free hyperpotassic solution). This activity may be related to mobilization of extracellular calcium. So this is an oxytocic-like action.

In different modified physiological media, the addition of the ESera caused the development of contracture in the presence of EGTA. This fact suggested that ESera could act on the double calcium flux as prostaglandin. This prostaglandin-like activity was summarized by the ability to mobilize intracellular and extracellular calcium, which confirmed the uterotonic property of ESera. Accordingly, this plant extract certainly possesses compounds of great interest in obstetrics and gynecology. Our study could justify the use of the leaves of *S. radiatum* as oxytocic-like in traditional medicine to facilitate childbirth.

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ABBREVIATIONS

ESera: *Sesamum radiatum* leaf aqueous extract; PRUPs, pregnant rat uterine preparations, OT: oxytocin; Miso: misoprostol; EGTA: ethyleneglycol-bis(aminoethyl ether) *N,N,N',N'*-tetraacetic acid; CF: contractile force; BT: basal tone; FC: frequency of contraction; EC_{50}: efficient concentration 50%.

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AUTHORS' CONTRIBUTIONS

All authors contributed equally in the study. They made substantial contributions to the design of the study, the collection of the data as well as the preparation and analysis of the data. They also drafted the manuscript and gave final approval for its submission to the journal for consideration of publication.

DECLARATION OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

REFERENCES

[1] D. Diallo, I. P. Guissou, M. Ha ïdara, C. Tall, and O. M. J. Kasilo, "Research on African traditional medicine: Hypertension," Afr. Health Monit. Vol. 14, pp. 58-63, 2010. (Published in French).

[2] M. T. Mbizvo, S. Fawcus, G. Lindmark, and L. Nystrom, "Maternal mortality in rural and urban Zinbabwe: Social and reproductive factors in an incident case-referent study," Soc. Sci. Med. Vol. 36, pp. 1197-1205, 1993.

[3] Bellomaria B, and P. Kacou, "Plants and popular medicine at Agboville (Ivory Coast)," Fitoterapia Vol. 66, pp. 117-141, 1995. (Published in French).

[4] K. N'Guessan, "The fight against the tragedy of delivery by plants," Rev. Inter. Sci. de la Vie et de la Terre Vol. 1, pp. 57-66, 2000. (Published in French).

[5] D. F. Malan, and D. F. R. Neuba, "Traditional practices and medicinal plants use during pregnancy by Anyi-Ndenye women (Eastern Côte d'Ivoire)," Afr. J. Reprod. Health Vol. 15, pp. 85-93, 2011.

[6] B. A. Konan, J. Y. Datté, and A. M. Offoumou, "Action of the aqueous extract of *Sesamum radiatum* Schum. and Thonn. (Pedaliaceae) on the cardiovascular system of mammalians: Hypotensive effect," Curr. Bioact. Compd. Vol. 2, pp. 263-267, 2006.

[7] B. A. Konan, J. Y. Datté, and A. P. Yapo, "Nitric oxide pathway-mediated relaxant effect of aqueous sesame leaves extract (*Sesamum radiatum* Schum. & Thonn.) in the guinea-pig isolated aorta smooth muscle," BMC Complent. Alter. Med. Vol. 8, Art. 23, 2008.

[8] J. Y. Datté, H. A. Traoré, A. M. Offoumou, and M. Vangah-Manda, "Antihypertensive effect of *Caesalpinia bonduc* (Caesalpiniaceae) aqueous leaf extract on arterial blood pressure of guinea-pig," Revue Med. Pharm. Afr. Vol. 11-12, pp. 79-88, 1997. (Published in French).

[9] J. C. Abo, K. J. Aka, E. E. Ehilé, F. Traoré, and F. Guédé-Guina, "Cholinergic effects of *Mareya micrantha* (Euphorbiaceae) crude extract on blood pressure and heart activity," Afrique Biomed. Vol. 5, pp. 11-20, 2000. (Published in French).

[10] A. Fourni é, A. Laffitte, O. Parant, and P. Ko-Kivok-Yun, "Modification in the maternal organism during pregnancy," Encycl Med Chir (Editions scientifiques et Medicales Elsevier SAS, Paris), Gynecologie/Obstetrique, 5-008-A-10, 8p, 1999. (Published in French).

[11] T. F. Baskett, V. L. Persad, H. J. Clough, and D. C. Young, "Misoprostol versus oxytocin for reduction of postpartum blood loss," Int. J. Gynaecol. Obstet. Vol. 97, pp. 2-5, 2007.

[12] H. Barata, M. Thompson, W. Zielinska, Y. S. Han, C. B. Mantilla, Y. S. Prakash, S. Feitoza, G. Siek, and E. N. Chini , "The role of cyclic-ADP-ribose-signaling pathway in oxytocin-induced Ca^{2+} transients in human myometrium cells," Endocrinology Vol. 145, pp. 881-889, 2004.

[13] F. Behnamfar, Sh. Nafisi, M. Behrashi, Z. Majidi, and Z. Sadat, "Effects of epidfural analgesia on labour course," Pak. J. Biol. Sci. Vol. 9, pp. 2378-2380, 2006.

[14] F. Sahhaf, A. Alizadeh, H. Kokcheli, and M. Ghojazadeh, "Effect of uterine contraction and amniotomy on fetal carditocograph," Pak. J. Biol. Sci. Vol. 13, pp. 34-39, 2010.

[15] S. Wray, K. Jones, S. Kupittayanant, Y. Li, A. Matthew, E. Monir-Bishty, K. Noble, S. J. Pierce, S. Quenby, and A. V. Shmygol, "Calcium signaling and uterine contractility," J. Soc. Gynecol. Investig. Vol. 10, pp. 252-264, 2003.

[16] S. I. H. Taqvi, M. N. Ghayr, A. H. Gilani, M. T. Aftab, and Z. S. Saify, "Synthesis, spasmolytic, cardio-suppressant, vasodilator and Ca++ antagonist activities of 1-[4'-methylphenacyl]-4-acetyl-4-phenylpiperidinium bromide," Pak. J. Biol. Sci. Vol. 9, pp. 60-66, 2006.

[17] S. Kupittayanant, P. Kupittayanant, and C. Suwannachat, "Mechanisms of uterine contractile in laying hens," Anim. Reprod. Sci. Vol. 115, pp. 215-224, 2009.

[18] A. Shmygol, J. Gullam, A. Blanks, and S. Thornton, "Multiple mechanisms involved in oxytocin-induced modulation of myometrial contractility," Acta Pharmacol. Sin. Vol. 27, pp. 827-832, 2006.

[19] Z. Tabasi, M. Behrashi, and M. Mahdian , "Vaginal misoprostol versus high dose of oxytocin for labor induction: a comparative study," Pak. J. Biol. Sci. Vol. 10, pp. 920-923, 2007.

[20] M. Behrashi, and M. Mahdian, "Varginal versus oral misoprostol for second-trimester pregnancy termination: A randomized trial," Pak. J. Biol. Sci. Vol 11, pp. 2505-2508, 2008.

[21] M. Mosihuzzaman, and M. I. Choudhary, "Protocols on safety, efficacy, standardization and documentation of herbal medicine," Pure Appl. Chem. Vol. 80, pp. 2195-2230, 2008.

[22] B. A. Konan, A. K. Amonkan, M. L. B. Ahui, G. M. K. Bouafou, L. K. Kouakou, F. Z. Kpahé, and J. Y. Datté , "Myostimulating effects of *Sesamum radiatum* aqueous leaf extract in isolated guinea-pig *Taenia caeci* contractile activity," Afr. J. Trad. Compl. Alter. Med. Vol. 8, pp. 377-385, 2011.

[23] J. Y. Datté, A. Traoré, A. M. Offoumou, and A. Ziegler, "Effects of leaf extract of *Caesalpinia bonduc* (Caesalpiniaceae) on the contractile activity of uterine smooth muscle of pregnant rats," J. Ethnopharmacol. Vol. 60, pp. 149-155, 1998.

[24] E. E. Bafor, E. K. Omogbai, and R. I. Ogulua, "Evaluation of the uterotonic activity of the aqueous leaf extract of *Ficus exasperata* Vahl (Moraceae)," Res. J. Med. Plant Vol. 3, pp. 34-40, 2009a.

[25] E. Johnson, M. Chuodhury, E. O. Abraham, and U. A. Sunday, "Pharmacological studies of bark of Commiphora Africana (Burseraceae)," J. Pharmacol. Toxicol. Vol. 7, pp. 52-57. 2012.

[26] A. H. Gilani, S. Mandukhail, J. Iqbal, M. Yasinzai, N. Aziz, A. Khan, and Najeeb-ur-Rehman, "Antispasmodic and vasodilator activities of *Morinda citrifolia* root extract are mediated through blockade of voltage dependent calcium channels," BMC Complent. Alter. Med. Vol. 10, Art. 2, 2010.

[27] A. J. Farre, M. Columbo, M. Fort, and B. Gutierrez, "Differential efects of various Ca^{2+} antagonists," Gen. Pharmacol. Vol. 22, pp. 177-181, 1991.

[28] W. D. H. Chiwororo, J. A. O. Ojewole, "Spasmolytic effect of *Psidium guajava* Linn. (Myrtaceae) leaf aqueous extract on rat isolated uterine horns," J. Smooth muscle Res. Vol. 45, pp. 31-38, 2009.

[29] J. Y. Datté, M. A. Offoumou, and O. Manda, "Uterotonic effects of hydromethanolic extract of *Parquetina nigrescens* (Periplocaceae) on spontaneous contractile activity in isolated myometrium of pregnant rat," J. Etnopharmacol. Vol. 53, pp. 15-20, 1996.

[30] F. Traoré, N. B. Mbongue, F. Guédé-Guina, P. P. Koné, "Uterotonic effect of an aqueous extract of *Mareya micrantha* (Euphorbiacee) on the spontaneous contractions of pregnant uterus from rat," Rev. Med. Pharm. Afr.Vol. 18, pp. 1-16, 2004. . (Published in French).

[31] M. A. Sattar, N. A. Abdullah, M. A. H. Khan, A. Dewa, and D. Samshia, "Uterotrophic, fetotoxic and abortifacient effect of a Malaysian variety of *Plumbago rosea* L. on isolated rat uterus and pregnant mice," Pak. J. Biol. Sci. Vol. 10, pp. 763-767, 2007.

[32] E. E. Bafor, M. Nwiko, E. K. I. Omogbai, R. I. Ozolua, Z. A. M. Nworgu, "Evaluation of the proposed inhibitoy

effect of the aqueous sterm-bark extract of *Ficus exasperata* on uterine preparations *in vitro*," Int. J. Pharmacol.Vol. 5, pp. 94-97, 2009b.

[33] G. J. Hofmeyr, A. M. Gulmezoglu, and Z. Alfirevic, "Misoprostol for induction of labor: A systematic review," Br. J. Obstet. Gynaecol. Vol. 106, pp. 798-803, 1999.

[34] D. Mota-Rojas, D. Villanueva-Garcia, M. Alonso-Spilsbury, M. Becerril-Herrera, R. Ramirez-Necoechea, M. Gonz âlez-Lozano, and M. E. T. Ortega, "Effect of different doses of oxytocin at delivery on suffering and survival of new born pigs," J. Med. Sci. Vol. 7, pp. 170-178, 2007.

[35] N. Moslemizadeh, T. G. Moghadam, S. Peyvandis, "Evaluation of vaginal misoprostol effect on pregnancy rate after intrauterine insemination," Pak. J. Biol. Sci. Vol. 12, pp. 64-68, 2009.

[36] P. Mostafa-Gharebaghi, M. Mansourfar, and H. Sadeghi-Bazargani, "Low dose vaginal misoprostol versus prostaglandin E2 suppository for early uterine evacuation: A randomized clinical trial," Pak. J. Biol. Sci. Vol. 13, pp. 946-950, 2010.

[37] T. Cherian, "Effect of papaya latex extract on gravid and non-gravid rat uterine preparation *in vitro*," J. Ethnopharmacol. Vol. 70, pp. 205-212, 2000.

[38] Y. Kasai, M. Iino, O. Tsutsumi, Y. Taketani, and M. Endo, "Effects of cyclopiazenic acid on rhythmic contractions in uterine smooth bundles of the rat," Br. J. Pharmacol. Vol. 112, pp. 1132-1136, 1994.

[39] P. K. Aley, H. J. Noh, X. Gao, A. A. Tica, E. Brailoiu, and G. C. Churchill, "A functional role for nicotinic acid adenine dinucleotide phosphate in oxytocin-mediated contraction of uterine smooth muscle from rat," J. Pharmacol. Exp. Ther. Vol. 333, pp. 726-735, 2010.

[40] W. Blair-Bell, and P. Hick, "Report CXII. Observations on the physiology of the female genital organs," Br. Med. J. Vol. 1, pp. 655-658, 1909.

[41] Q. Jabeen, N. Aziz, Z. Afzal, A. H. Gilani, "The spasmogenic and spasmolytic activities of Lavandula stoechas are mediated through muscarinic receptor stimulation and calcium channel blockade," Int. J. Pharmacol. V

[42] K. Noble, A. Matthew, T. Burdyga, and S. Wray, "A review of recent insights into the role of the sarcoplasmic reticulum and Ca entry in uterine smooth muscle," Eur. J. Obstet. Gynecol. Reprod. Biol. Vol. 144, pp. S11-S19, 2009.

[43] J. Mironneau, "Effects of oxytocin on ionic currents underlying rhythmic activity and contraction in uterine smooth muscle," Pflugers Arch. Vol. 363, pp. 113-118, 1976.

[44] K. Tasaka, N. Masumoto, A. Miyake, and O. Tanizawa, "Direct measurement of intracellular free calcium in cultured human puerperal myomotrial cells stimulated by oxytocin: effects of extracellular calcium blockers," Obstet. Gynecol. Vol. 77, pp. 101-106, 1991.

[45] S. Batra, "Uptake and energy-dependent extrusion of calcium in the rat uterus," Acta Physiol. Scand. Vol. 114, pp. 447-452, 1982.

[46] L. Hurwitz, D. F. Fitzpatrick, G. Debbas, and E. J. Landon, "Localization of calcium pump activity in smooth muscle," Science Vol. 179, pp. 384-386, 1973.

[47] T. W. Wilson, and D. W. Quest, "Comparative pharmacology of calcium antagonists," Can. J. Cardiol. Vol. 11, pp. 243-249, 1995.

[48] M. Kawanishi, K. Baba, and T. Tomita, "Effects of sodium removal and readmission on the mechanical response in the guinea-pig tracheal smooth muscle," Jap. J. Physiol. Vol. 34, pp. 127-139, 1984.