



# Uterotonic activity of an aqueous extract of the leaves of *Helichrysum mechowianum* commonly used for vaginal tightening by native populations in south east Gabon

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## Abstract

The leaves of *Helichrysum mechowianum* (Helymext) are traditionally used by some native populations of Gabon for vaginal tightening following childbirth. In order to validate its use scientifically, the current work was undertaken to investigate the possible tonic activity of the plant in the vaginal smooth muscles. Phytochemical screening of the plant extracts was carried out and its pharmacological effects were studied on isolated uterine smooth muscles from pregnant rats using the isolated organ bath. Helymext ( $10^{-8}$  -  $10^{-2}$  mg/ml) evoked a concentration-dependent enhancement of the uterine smooth muscle contractile activity ( $EC_{50}$ :  $1.34 \times 10^{-4} \pm 0.1 \times 10^{-5}$  mg/ml) comparable to that induced by acetylcholine (ACh) ( $EC_{50}$ :  $9.41 \times 10^{-7} \pm 0.02 \times 10^{-8}$  mg/ml) or by prostaglandin (PGE1) ( $EC_{50}$ :  $8.46 \times 10^{-7} \pm 1.21 \times 10^{-9}$  mg/ml). Atropine ( $10^{-6}$  mg/ml) failed to inhibit Helymext-induced contractions ( $EC_{50}$ :  $4.4 \times 10^{-4} \pm 0.12 \times 10^{-5}$  mg/ml,  $p > 0.05$ ). Whereas, indomethacin ( $10^{-6}$  mg/ml) significantly reduced the contractile activity caused by the plant extract ( $EC_{50}$ :  $9.68 \cdot 10^{-5} \pm 0.2 \times 10^{-4}$  mg/ml,  $p < 0.05$ ). In calcium-free medium with or without EDTA, Helymext still induced spontaneous and tonic contractions. In the same conditions, a tonic contraction was caused by the PGE1. These results suggest that *Helichrysum mechowianum* plant extract would contain uterotonic ingredients which could act through pathways involving prostaglandin synthesis. Helymext-induced calcium mobilization from extracellular medium and from internal stores, highlighting a great potential of enhancement of the smooth muscle tonic activity.

**Key words:** Herbal medicines, *Helichrysum mechowianum*, Uterotonic, Vaginal tightening

## 1. Introduction

Herbal medicines are commonly used by women in sub-Saharan Africa, both in urban and rural areas for the management of gynaecological conditions and disorders associated with the female reproductive system as done by several women worldwide [1, 2]. Indeed, many plants such as *Caesalpinia bonduc* [3], *Clivia miniata* [4] and *Ficus asperifolia* [5] are used for their uterotonic properties to induce labour.

In Gabonese pygmy population, in which herbal medicine is widely used, many plants are used during pregnancy, childbirth, and post-partum period mainly to promote the restoration of the female genital tract, especially for the vaginal tightening. Among them, *Helichrysum mechowianum* is commonly used by females from villages of the M'passa department, in South Eastern Gabon, to promote vaginal rejuvenation.

It is well documented that physiological mechanisms involved in the structural and physiological recovery following childbirth is due to the secretion of oestrogen which induces a tightening of the vaginal walls, tissue turnover, and increases vaginal lubrication, tonicity and

elasticity [6]. Thus, the cosmetic vaginal reconstruction surgery is increasingly used in developed countries [7].

*H. mechowianum* belonging to the botanical family of Asteraceae is used for many disorders including abdominal pain, fever, cough, gastric ulcers, acute hepatitis and female sterility [8]. It is known to exhibit antibacterial activity [8]. Phytochemical studies revealed the presence of several bioactive compounds such as flavonoids, phloroglucinols,  $\alpha$ -pyrones, coumarins and terpenoid compounds [8]. In order to scientifically validate the use of *H. mechowianum* in the improvement of sexual life through the vaginal tightening, we studied the effect of the plant extract on the restoration of uterus tone.

## 2. Material and methods

### 2.1. Plant material

Leaves of *Helichrysum mechowianum* (Asteraceae) were collected in surrounding villages of the M'Passa department in the Haut-Ogooué province of south east Gabon. The leaves were collected in the villages of Benguia 2 and of Moulendé Colas, from an area consisting of a mosaic of savannah and forest. The plant material was authenticated

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by Yves ISSEMBE, an expert botanist of the Gabon National Herbarium. A voucher specimen (n° 638) was deposited in the herbarium in the Faculty of Sciences (University of Sciences and Techniques of Masuku).

## 2.2. Animals

Animals used were female pregnant Wistar rats weighing between 150 and 200 g. They were provided by the Animal Laboratory of the National Institute of Traditional Medicine and Pharmacopoeia (IPHAMETRA). Animals were housed at a constant room temperature with a light/dark cycle of 14/10 hr. They were fed and given water ad libitum. Experimental protocols were performed in accordance with the care and use of Laboratory animals in Gabon.

## 2.3. Drugs

The drugs used, acetylcholine (ACh), atropine (ATR), indomethacin (Indo), L-NAME, EDTA, were purchased from de Sigma–Aldrich (Germany) and Misoprostol (PGE1) was from Glasgow laboratories(France).

## 2.4. Preparation of the plant extract

Fresh leaves of *Helichrysum mechowianum* were harvested in the field. They were dried at room temperature in the laboratory. The air-dried leaves were reduced into powder with a mortar. Next, the powder (60 g) was macerated in 600 ml of double distilled water for 24 hr under magnetic stirring. The macerate was successively filtered on cotton and Wattman filter paper. The filtrate was evaporated using the BUCHI Rotavapor® at 40 °C. The plant extract obtained (Helimext) was stored at 4 °C until use.

## 2.5. Phytochemical screening

The aqueous extract of the plant was subjected to qualitative chemical screening for the identification of the tannins, alkaloids and flavonoids using standard procedures [9].

## 2.6. Contractile activity of the uterine smooth muscle

The animal was sacrificed by cervical dislocation and a midline laparotomy was performed to remove the pregnant uterus. The uterus was isolated and pieces of 1 to 1.5 mm were removed and placed in a petri dish containing the Mac Ewen physiological solution (130 mM NaCl, 5.63 mM KCl, 5.52 mM CaCl<sub>2</sub>, 0.93 mM H<sub>2</sub>PO<sub>4</sub>Na, 11.9 mM HCO<sub>3</sub>Na, 0.24 mM MgCl<sub>2</sub> and 0.24 mM glucose). Isolated uterine strips were suspended in the organ bath with one end connected to an isometric force transducer (F30 HSE 372, Hugo Sachs Elektronik, March-Hugstetten, Germany). Isometric tension was amplified using an amplifier (D-79232 March, Hugo Sachs Electronic, Germany) and mechanic responses were recorded on a Rikadenki multi-pen recorder (R-64-D, Rikadenki Co., Ltd, Japan). Uterine strips were equilibrated for 90 min under a resting tension of 1 g. Dose-response effects were recorded for the aqueous

extract and control drugs until a plateau was reached. The effects of the extract were compared to those of the standard drugs in normal physiological solution as well as in calcium-free solution in the presence or in the absence of EDTA.

## 2.7. Data analysis

The INSTAT 3 software was used to perform the statistical analysis using One-way ANOVA followed by multiple comparison test, Dunnett's t-test. Data were expressed as the mean ± S.E.M. The GraphPad Prism 5 (San Diego, CA, USA) software was used to achieve the graphical representations of data. *p* values less than 0.05 were considered as statistically significant.

## 3. Results

### 3.1. Phytochemical constituents

Table 1 shows the presence of various secondary metabolites revealed by the phytochemical screening in the leaves of *H. mechowianum*.

Table 1. Phytochemical screening of aqueous extract of *H. mechowianum* leaf

Chemical compounds	Aqueous extract
Saponins	-
Gallic tannins	+++
Catechic tannins	+++
Anthraquinones	++
Total phenols	+++
Total flavonoids	-
Flavanols	++
Flavones	-
Flavanones	-
Coumarins	+++
Digitoxin	+
Digitoxigenin	-
Gitoxin	-
Gitoxigenin	-
Alkaloids	-
Sterols and triterpenes	+++
Reducing compounds	++

Note: + indicates presence and – indicates absence

A strong presence of tannins, total phenols, coumarins, sterols and triterpenes was observed in the crude aqueous extract. Anthraquinones and flavanols were also detected.

### 3.2. Effects of Helymext on the contractile activity of isolated rat uterus

The effects of increasing concentrations of Helymext (10<sup>-8</sup> - 10<sup>-2</sup> mg/ml) in the uterine contractions are depicted in Figure 1.

The tension of spontaneous contractions recorded under control conditions was 8.37 mN. Helymext induced an increase in the smooth muscle spontaneous contractions with a maximal tension of  $12.35 \pm 0.9$  mN obtained at a

uterus. Increasing concentrations of PGE1 ( $10^{-8}$  -  $10^{-2}$  mg/ml) and those of ACh ( $10^{-8}$  -  $10^{-2}$  mg/ml) enhanced the contractions of the isolated organ (Fig. 2a and 2b). Indeed, as indicated by the curves (Fig. 2c and d), ACh or PGE1, in

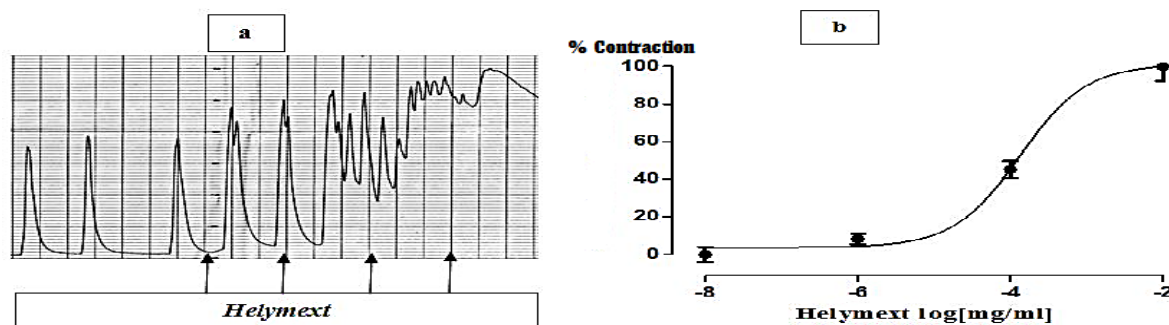


Figure 1. Effect of aqueous extract of *Helichrysum mechowianum* (Helymext) on the rat uterine contractile activity. (a) Recording of the contractile activity induced in the absence and in the presence of increasing concentrations of Helymext. The arrows indicate the application of increasing concentrations of substances ( $10^{-8}$  to  $10^{-2}$  mg/ml, n=3). Horizontal Scale: 4 min; Vertical scale: 0.5 mN. (b) Sigmoidal curve indicating a dose-dependent responses elicited by Helymext ( $10^{-8}$  to  $10^{-2}$  mg/ml).

concentration of  $10^{-2}$  mg/ml (Fig.1a). A development of tonic contractions was observed at a concentration of  $10^{-6}$  mg/ml. The figure 1b shows the dose-dependent responses evoked by the plant extract with an EC<sub>50</sub> of  $1.34 \times 10^{-4} \pm 0.1 \times 10^{-5}$  mg/ml.

### 3.3. Effect of PGE1 and ACh on the contractile activity of the isolated rat uterus

Figure 2 shows the effects of standard drugs (PGE1 and ACh) on the spontaneous contractions of the isolated rat

the concentration range of  $10^{-8}$  mg/ml to  $10^{-2}$  mg/ml, elicited a dose-dependent increase in contractile force. Significant increases ( $p < 0.01$ ) in the contractile forces of ACh and PGE1 were observed at a concentration of  $10^{-6}$  mg/ml with EC<sub>50</sub> of  $9.41 \times 10^{-7} \pm 0.02 \times 10^{-8}$  mg/ml and of  $8.46 \times 10^{-7} \pm 1.21 \times 10^{-9}$  mg/ml respectively.

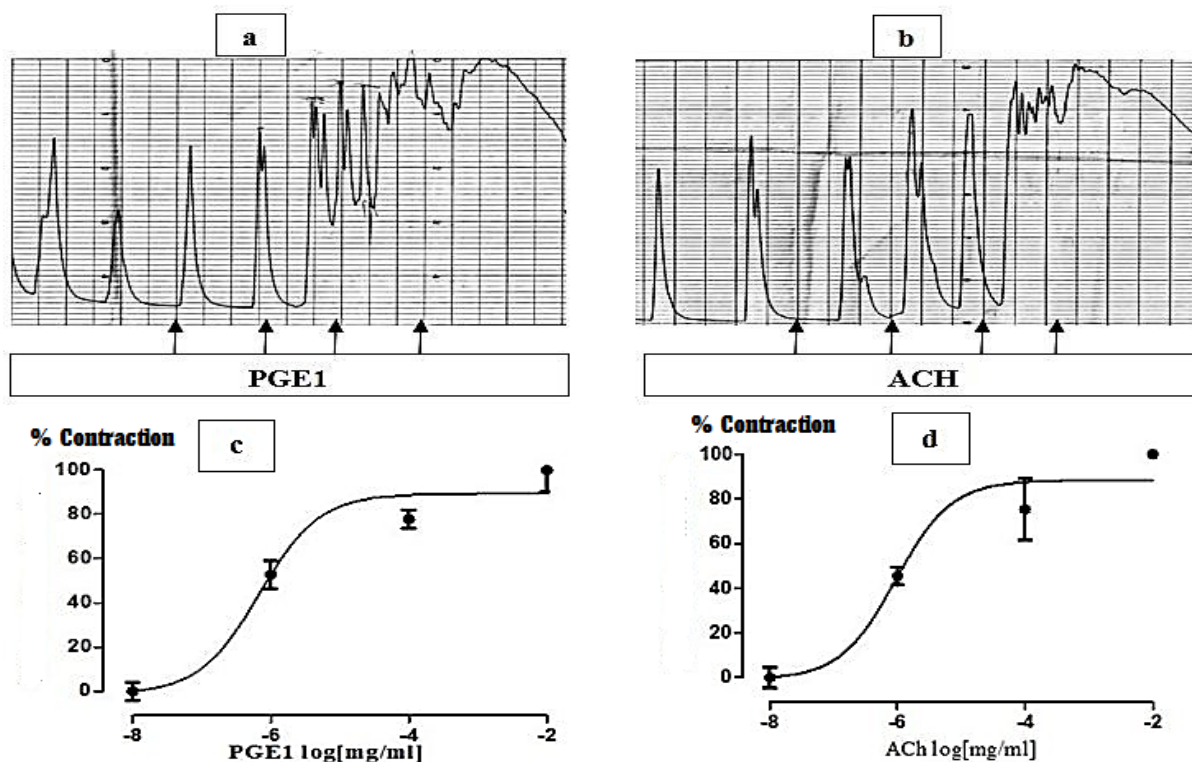


Figure 2. Effects of misoprostol (PGE1) and of acetylcholine (ACh) on isolated rat uterine contractions. (a) and (b): Recording of the contractile activities induced by PGE1 (a) and ACh (b) respectively in the absence and in the presence of these standard drugs. The arrows indicate the application of increasing concentrations of substances ( $10^{-8}$  to  $10^{-2}$  mg/ml, n=3). Horizontal scale: 4 min, vertical scale: 0.5mN. (c) and (d): Dose-dependent response curves induced by PGE1 (c) and by ACh (d).

### 3.4. Effects of Helymext and ACh on the contractile activity of the isolated rat uterus in the presence of atropine

In figure 3, panel 3a and 3b show, respectively, the effects of ACh and of Helymext on the contractile activity of the myometrium pretreated with atropine. Figure 3a

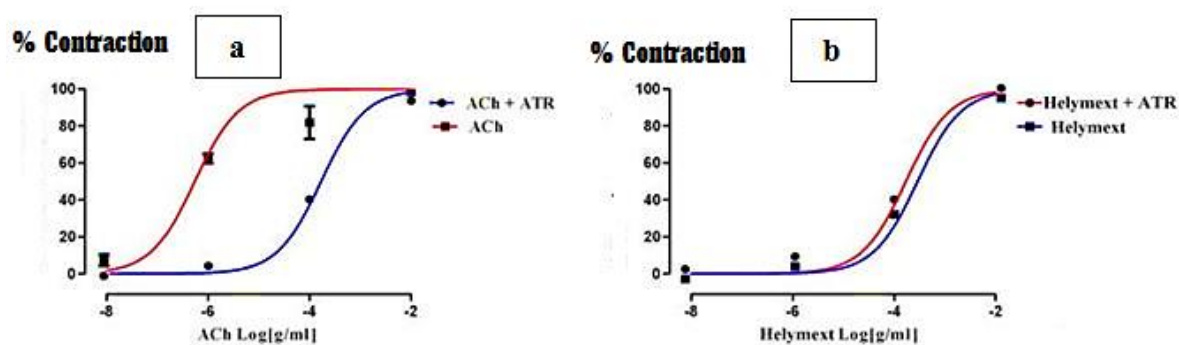


Figure 3. Dose-dependent response curves induced by ACh ( $10^{-8}$  -  $10^{-2}$  mg/ml) and by Helymext ( $10^{-8}$  -  $10^{-2}$  mg/ml) in the absence and in the presence of atropine ( $10^{-6}$  mg/ml) (n=3).

shows a dose-dependent increase in the contractile force of the myometrium induced by ACh ( $10^{-8}$  -  $10^{-2}$  mg/ml), in the absence and the presence of atropine ( $10^{-6}$  mg/ml).

A shift to the right of the dose-dependent curve of the ACh-induced contractions was observed in the presence of atropine with a significant difference between the respective EC<sub>50</sub> ( $9.41 \times 10^{-7} \pm 2.1 \times 10^{-9}$  mg/ml vs  $2.48 \times 10^{-6} \pm 2 \times 10^{-10}$  mg/ml,  $p < 0.01$ ) indicating a significant inhibition of contractile activity of ACh. Whereas, no significant changes were observed in the EC<sub>50</sub> obtained with Helymext in the absence or in the presence of atropine (EC<sub>50</sub>:  $1.34 \times 10^{-4} \pm 0.1 \times 10^{-5}$  mg/ml vs  $4.4 \times 10^{-4} \pm 0.12 \times 10^{-5}$  mg/ml,  $p > 0.05$ ).

### 3.5. Effects of Helymext and misoprostol (PGE1) on the contractile activity of the isolated rat uterus in the presence of indomethacin

induced a dose-dependent increase in the contractile force of the myometrial smooth muscle in the absence as well as in the presence of indomethacin.

However, a shift to the right in the concentration curve of PGE1 was observed when the drug was applied in the presence of indomethacin with a significant difference between the respective EC<sub>50</sub> ( $8.46 \times 10^{-7} \pm 3 \times 10^{-9}$  mg/ml vs

$8.29 \times 10^{-5} \pm 1.3 \times 10^{-8}$  mg/ml,  $p < 0.01$ ). A similar effect was observed with Helymext when administered in the absence and presence of indomethacin with a significant difference between the EC<sub>50</sub> (EC<sub>50</sub>:  $1.34 \times 10^{-4} \pm 0.1 \times 10^{-5}$  vs  $9.68 \times 10^{-5} \pm 0.2 \times 10^{-4}$  mg/ml,  $p < 0.05$ ).

### 3.6. Effects of PGE1 and Helymext in the contractile activity of uterine smooth muscle in the calcium-free medium ( $0Ca^{2+}$ ).

The figure 5 (a and b) shows the effects of PGE1 and Helymext in calcium-free medium. In a calcium-free medium ( $0Ca^{2+}$ ) a total suppression of the contractile activity of the uterine contractions was observed.

When applied in this medium, PGE1 ( $10^{-5}$  mg/ml) exhibited a development of a tonic contraction with a maximal tension of  $3.06 \pm 0.7$  mN. Under the same

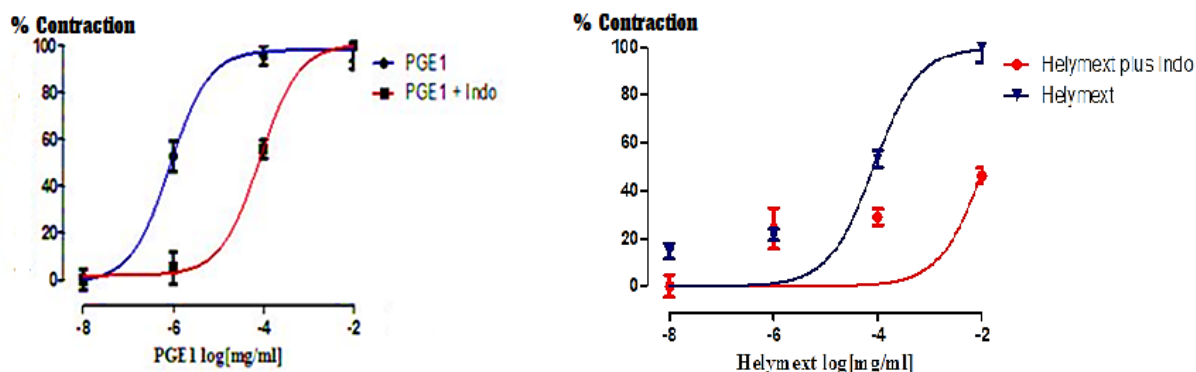


Figure 4. Effects of PGE1 ( $10^{-8}$  -  $10^{-2}$  mg/ml) and Helymext ( $10^{-8}$  -  $10^{-2}$  mg/ml) in the absence and in the presence of indomethacin ( $10^{-6}$  mg/ml).

The figure 4 shows the effects of PGE1 ( $10^{-8}$  -  $10^{-2}$  mg/ml) and of Helymext ( $10^{-8}$  -  $10^{-2}$  mg/ml) on the contractile activity of the myometrium, in the absence and in the presence of indomethacin ( $10^{-6}$  mg/ml). PGE1

conditions, Helymext ( $10^{-4}$  mg/ml) caused an increase in the



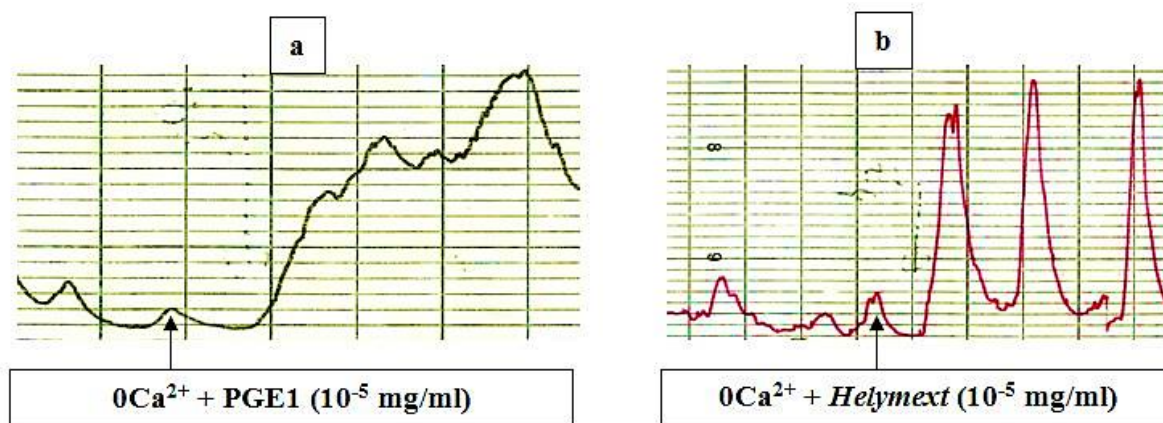


Figure 5. Effects of PGE1 (5a) and of Helymext (5b) on the contractile activity of the isolated rat uterine smooth muscle in a calcium-free medium ( $0Ca^{2+}$ ); Horizontal Scale: 4 min; Vertical scale: 0.5 mN.

basal tone and development of spontaneous contractions with a maximal tension of  $4.95 \pm 0.17$  mN.

### 3.7. Effects of PGE1 and Helymext on the contractile activity of uterine smooth muscle in the totally calcium-deprived medium ( $0Ca^{2+} + EDTA$ ).

In a totally calcium-deprived medium, by adding EDTA at a concentration of  $10^{-6}$  mg/ml ( $0Ca^{2+} + EDTA$ ), a suppression of the spontaneous contractile activity of the myometrium was recorded (Fig. 6a and b).

PGE1 ( $10^{-5}$  mg/ml) induced a development of a tonic contractions maintained at a magnitude of 1.89 mN. Under the same conditions, Helymext ( $10^{-4}$  mg/ml) induced an

stimulate labour such as, *Ficus deltoidea* [2] *Clivia miniata* [4] and *Ficus asperifolia* [5]. These results suggest that the aqueous extract of *Helichrysum mechowianum* (Helymext) may contain uterotonic ingredients which could mimic the action of some well-known uteroactive agents.

Among the well-established uterotonic agents, oxytocin is known as the most commonly used and most potent [1, 10] followed by prostaglandins [11, 12, 13, 14], acetylcholine [5, 14], and histamine [2, 15]. The effects of acetylcholine and those of prostaglandin E1, used as the reference drugs, are respectively inhibited by muscarinic receptor antagonist such as atropine [16], and by prostaglandin synthesis inhibitor, such as indomethacin [17] or by prostaglandin receptor inhibitor [18]. It was shown

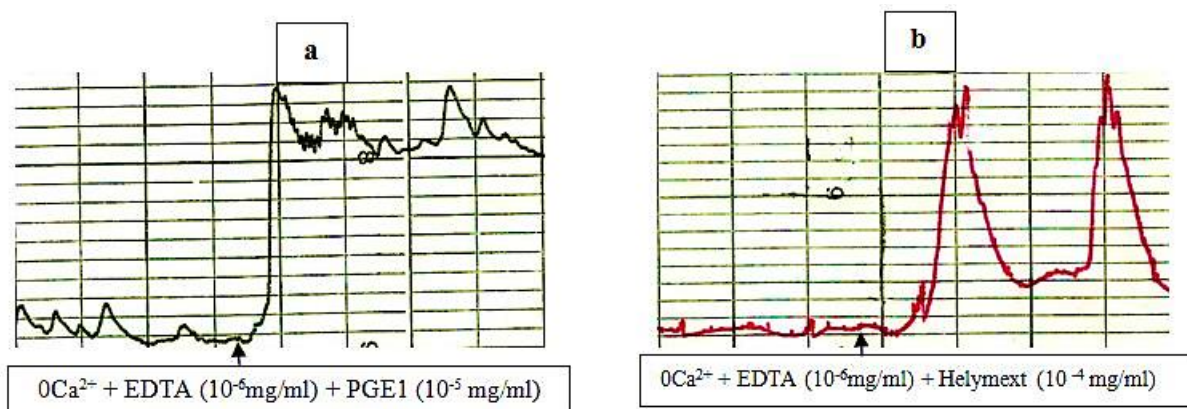


Figure 6. Effects of PGE1 (a) and of Helymext (b) on the contractile activity of isolated rat uterine smooth muscle in totally calcium-deprived medium.

increase in the basal tone and the development of spontaneous contractions with an amplitude of 2.49 mN.

## 4. Discussion

The study of the effects of *Helichrysum mechowianum* in isolated rat uterus showed a concentration-dependent increase of uterine contraction comparable to those evoked by acetylcholine or by prostaglandin. This effect was also similar to that reported on a great number of uterotonic medicinal plants used in traditional medicinal system to

that the contractile responses caused by Helymext were not inhibited by atropine, a non-selective muscarinic receptor antagonist [19]. Whereas indomethacin, a prostaglandin synthesis inhibitor, significantly decreased Helymext-induced contractions. This finding suggests that the cholinergic mechanisms may not be involved. Indeed, it is well established that the Ach-induced uterine contractions are due to the stimulation of myometrial muscarinic receptors [5, 19]. On the other hand, based on the decrease of plant extract's effects obtained in the presence of indomethacin, pathways involving prostaglandin synthesis, such as oxytocin may be involved in the mechanism of

Helymext-induced contractions. Indeed, it is well known that oxytocin, a potent uterotonic hormone [1, 2], act both directly through myometrial oxytocin receptors (OT1a) to cause uterine contraction, and indirectly through endometrial oxytocin receptors (OT1b) to stimulate prostaglandin synthesis leading to the uterine contractions [2, 5]. Regarding the latter pathways, oxytocin is known to stimulate an increase in cytoplasmic phospholipase A2 (PLA2) and in cyclooxygenase 2 (COX2), the two major prostaglandins biosynthesis enzymes [1]. The prostaglandins-induced contractions are due to the activation of their receptors (EP1, EP3, FP) leading to the mobilization of  $[Ca^{2+}]_i$  from sarcoplasmic reticulum [20, 21]. The active compounds in Helymext may act through oxytocin receptors or a direct stimulation of prostaglandin receptors may also be involved. The ongoing studies, will allow us to clarify this hypothesis.

The calcium concentration ( $Ca^{2+}$ ), plays a key role in the smooth muscle contraction [1, 20]. The  $Ca^{2+}$  responsible for uterine smooth muscle contractions may be derived from extracellular medium and/or from intracellular calcium stores. When we compared the effects, in  $Ca^{2+}$  free medium, of PGE1 (which mobilises  $Ca^{2+}$  from intracellular stores to induce uterine tonic contractions) with those of helymext, a tonic contraction was observed with spikes of spontaneous contractions in the calcium-free medium as well as in the  $Ca^{2+}$  totally deprived medium containing the EDTA, a non-selective calcium chelator [11, 22]. This observation suggests that the mechanisms underlying the Helymext-induced uterotonic effects may involve both transmembrane calcium influx and the mobilization of intracellular calcium from internal stores.

However,  $Ca^{2+}$  from extracellular medium could have been provided by aqueous extract of *H. mechowianum* leaves, since spontaneous contractions were still induced in totally calcium-deprived medium. Thus, the leaves of *H. mechowianum* offer a great potential in enhancing uterine smooth muscle tonic activity which may support the use of the plant in vaginal tightening.

Phytochemical screening of the plant crude extract revealed the presence of tannins and flavanols, which were known to exert uterotonic effects. It is reported that flavonoids act on estrogen receptors and stimulate uterine contractions [23, 24, 25]. The plant extract-induced uterine contractions may be due to presence of these bioactive compounds.

## 5. Conclusion

Our study revealed that the aqueous extract of *H. mechowianum* contains phytochemical constituents with potential uterotonic effects. These active compounds would exhibit this effect by acting via prostaglandinergic mechanism of action and would mobilize both extra and intracellular calcium from internal stores. The plant extract may also provide calcium in the external medium. In addition to the phytoconstituents, the plant extract-induced tonic effect may justify its use for vaginal tightening. Ongoing in-vivo and ex-vivo studies will further elucidate

the mechanism of action of *H. mechowianum* extract and will allow us to identify the bioactive compounds involved.

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