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# Mechanism of action of *Pterorhachis zenkeri* (Meliaceae), a sexual enhancer: effect on vas deferens contractility

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Historic

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**Abstract**

Background: *Pterorhachis. zenkeri* commonly known as "Ayilalou" or "Démareur" is an aphrodisiac plant used by Cameroonian traditional healers to enhance libido and sperm production. This study was undertaken to investigate the aphrodisiac mechanism of *P. zenkeri* on the contractile activity of vas deferens with regard to its function on ejaculation. Materials and Methods: The proximal parts of rat vas deferens were mounted in a 20 mL organ bath containing Krebs solution at 37°C. Cumulative dose-response curves were recorded with KCl (35-280 mM), adrenaline (7-70 µM) and aqueous extract of *P. zenkeri* (3,55-21,3 mg/mL). Prazosin (type 1 alpha-adrenergic receptor antagonist, 10 µM), Nifedipine (a specific L-type calcium channel antagonist, 5 µM), 2-AminoethoxydiPhenyl Borate (2-APB, IP3 receptor antagonist, 200 µM) and calcium-free medium were further used to study the mechanism of action.
Results: KCl, adrenaline and aqueous extract of *P. zenkeri* showed a concentration-dependent effect on vas deferens contractibility. Nifedipine and Pazosin partially inhibited the effects of *P. zenkeri*. In calcium-free medium containing Ethylene Glycol-bis (2-aminoethylether)-N,N,N'N'-tetraacetic acid (EGTA, 1µM), *P. zenkeri* triggered vas deferens contraction which was completely abolished in the presence of 2-APB. These results suggest that the aqueous extract of *P zenkeri* possesses bioactive compounds capable of inducing vas deferens contractions through type 1 alpha adrenergic receptors and mobilization of extra and intracellular calcium.
Conclusion: These results confirm the aphrodisiac properties of *P. zenkeri* through the activation of the emissive phase of ejaculation.

## 1. Introduction

Smooth muscle cells form a continuous layer that lines the walls of the hollow organs such as blood vessels, intestines, urinary bladder, airways, lymphatics, penis, and uterus [1]. The contraction of the smooth muscles can be initiated by an increase in intracellular calcium. The mobilization of calcium in the intracellular medium can be done by various signaling pathways including adrenergic, purinergic and dopaminergic pathways [2]. Signaling molecules such as inositol 1,4,5-trisphosphate and cyclic ADP-ribose play pivotal roles in the control of intracellular calcium concentration. Stimulation of smooth muscle cells with contractile agonists like adrenalin whose receptors are coupled to G proteins leads to the activation of phospholipase C (PLC), which in turn initiates the phosphatidylinositol turnover and the production of IP3 and 1,2-diacylglycerol in smooth muscle cells. IP3 formed in smooth muscle cells upon agonist stimulation mediates the release of calcium from sarcoplasmic reticulum through IP3 receptors [2]. In male sexual organs, the contractile property of smooth muscles of vas deferens, seminal vesicle, and prostate plays an important functional role in the physiological mechanism of ejaculation.
Ejaculation is a physiological process consisting of two main phases: emission and expulsion.

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The first step in the emission phase is the closure of bladder neck, followed by the ejection of prostatic secretions mixed with spermatozoa from the vas deferens into prostatic urethra. Expulsion follows emission and refers to the ejection of semen through the urethral meatus [3]. During the emission phase of ejaculation, the sperm stored in the cauda epididymis and secretions from sexual accessory glands are propelled into the urethra by contractions of the smooth muscles of cauda epididymis, vas deferens, seminal vesicle, and prostate. Contractions of the smooth muscles of these organs are mainly due to the extracellular calcium influx through L-type voltage-dependent calcium channels [4]. L-Type calcium channels are members of voltage-dependent calcium channel family, which are pharmacologically recognized by their high sensitivity to a heterogeneous class of organic substances collectively known as calcium channel blockers such as dihydropyridines (Nifedipine). Ejaculatory function can be improved by aphrodisiacs which are substances that arouse sexual instinct, bring on desire or increase sexual pleasure or performance [5]. *P. zenkeri* knows as "Ayilalou" or "Démareur" is an aphrodisiac plant used in the South-West Region of Cameroon. The roots of this plant are claimed to enhance libido and sperm production [6]. Recent unpublished study confirmed its ability to improve sexual desire and performance. To the best of our knowledge, no work has been done with this plant on the physiology of the vas deferens, a structure involved in the emissive phase of ejaculation. The present work was therefore carried out to investigate the *in vitro* effects of the

aqueous extract of the dried roots of *P. zenkeri* on the contractility of rat vas deferens, a study which could permit us to partially postulate the implication of the plant in the handling of some male reproductive disturbances such as infertility.

## 2. Materials and methods

### 2.1. Plant material and preparation of aqueous extracts

Fresh roots of *P. zenkeri* were collected in the Center region of Cameroon (Makénéné: latitude 4.88556, longitude10.7947 with a savanna climate). Botanical authentication (21553 / SRF / Cam.) was done at the National Herbarium of Cameroon. Fresh roots of *P. zenkeri* were cut, shade-dried (ambient temperature 22-25 degrees) and powdered. The powder obtained was used for the preparation of aqueous extract. Therefore, 150 g of powder was macerated in 1000 mL of distilled water for 72 hours. After filtration, the filtrate was oven-dried (45°C) and 24.96 g of extract was obtained giving an extraction field of 16,64 %. The filtrate (56°C) to obtain 21 g of aqueous extract (AE)

### 2.2. Animals

In the present study, male albino rats (3 months old, 200–250 g, body weight) were used. They were obtained from the animal house of the Faculty of Science, University of Dschang, Cameroon. The animals were maintained in a standard environment (22–25°C; approximately 12 h of light and 12 h of dark cycle) and had food and water ad libitum. This study was approved by the scientific committee of the Department of Animal Biology, University of Dschang, which follows the internationally accepted standard ethical guidelines for laboratory animal use and care, as described in the European Economic Community guidelines (EEC Directive 86/609/EEC, of the 24$^{th}$ November, 1986).

### 2.3. Drugs

The following substances were purchased from Sigma Chemical (St Louis, MO, USA): Prazosin, potassium chloride (KCl), EGTA (Ethylene Glycol-bis (2-aminoethylether)-N,N,N'N'-tetraacetic acid) and 2-APB (2-AminoethoxydiPhenyl Borate). Nifedipine and adrenaline were purchased from a local pharmacy in the city of Dschang (Cameroon). All solutions were prepared using distilled water except 2-APB that was dissolved in dimethylsulfoxide (DMSO) and distilled water. Maximal concentration of DMSO in bathing solution (0, 1%) had no effect on vas deferens contraction.

### 2.4. Experimental design

#### 2.4.1. Isolation of vas deferens fragments

Rats were sacrificed by cervical dislocation. The vas deferens were promptly removed, cleared of connective tissues, and cut into two parts as to obtain a proximal portion nearer to the epididymis and a distal portion nearer to the sex accessory complex. The proximal part was used in the study and mounted in an organ bath of 20 mL capacity containing fresh Krebs solution of the following composition (mM/l): NaCl 115, $NaHCO_3$ 25, $CaCl_2$ 2.5, KCl 4.7, $MgCl_2$ 1.2, $KH_2PO_4$ 1.2, and D-glucose 11. The medium was maintained at 37 ± 0.5°C and continuously bubbled with air. The preparation was allowed for equilibration within 45 minutes during which the bathing solution was changed every 15 minutes.

#### 2.4.2. Drugs challenges

After equilibration, cumulative concentrations of KCl (35, 105, 175, 210, 245, 280 mM), adrenaline (7, 14, 28, 42, 56, and 70 µM) and aqueous extract of *P. zenkeri* (3.55, 7.1, 10.65, 14.2, 17.75, and 21.3 mg/mL) were recorded. The different concentrations of each agonist were administered at 5 minutes intervals. These experiments were reproduced 5 times with different fragments for each drug/extract concentration regarding the protocol used by Watcho et., al [12]. Results were expressed as contraction gram force (gf) for each substance.

#### 2.4.3. Determination of the mechanism of action of *P. zenkeri*

To determine the mechanism of action of *P. zenkeri*, the tissue was pre-incubated for 30 min with Prazosin (10 µmol) or Nifedipine (5 µmol) before administration of adrenaline, KCl or plant extract. The effects of adrenaline and plant extract were tested in a free calcium medium. The calcium-free (CF) Krebs solution was prepared by substituting $CaCl_2$ with EGTA (1 mmol) as described by Amobi [7]. Furthermore, plant extract was incubated in the presence of 2-APB (200 µmol) to investigate the involvement of the intracellular and extracellular calcium in the plant activity. The results were expressed as inhibition percentage and calculated as follows:

Inhibition% = (CF without antagonist-CF in the presence of antagonist) /(CF without antagonist) × 100.

#### 2.4.4. Statistical analysis

Data was expressed as means ± SEM. One-way analysis of variance (ANOVA) followed by Tukey HSD post hoc were used to assess statistical difference among groups. The results were significantly different when $p< 0.05$.

## 3. RESULTS

### 3.1. Effects of potassium chloride (KCl) and adrenaline on the contractility of vas deferens

Cumulative administration of KCl (35, 105, 175, 210 and 280 mM) resulted in a significant ($P<0.001$) (Table I) and concentration-dependent increase of the contraction force of the vas deferens compared to 35 mM. The maximum contraction force was 0.91 gf (Fig. 1). In the presence of adrenaline, concentration-dependent contractions of the organ were registered (Fig 1), the increase of the contraction forces was significant ($P<0.005$- $0.001$) compared to the lowest concentration. This strength ranged from 0.38 gf to 1.13 gf (Table I)

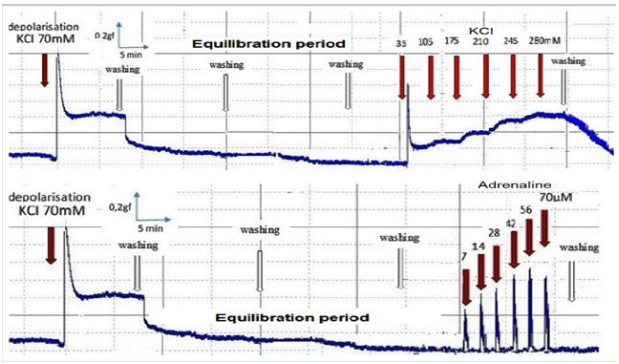


**Figure 1:** Effects of potassium chloride (KCl) and adrenaline on the contractility of vas deferens

### 3.2. Effect of *P. zenkeri* on the contractility of vas deferens

Similar to KCl and adrenaline, plant extracts induced contractions of vas deferens (Fig. 2) with maximal force of 1.36 gf obtained at 21.3 mg/mL (Table 4).

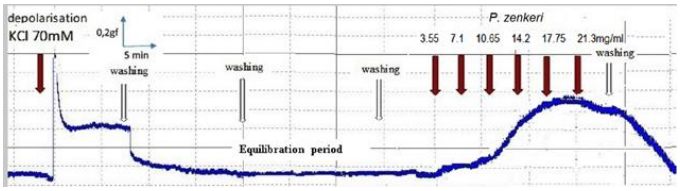


**Figure 2:** Effect of *P. zenkeri* on the contractility of vas deferens

**Table 1:** Effects of potassium chloride (KCl) and adrenaline on the contractility of vas

| Samples | Concentrations | Mean contraction forces (gf) |
|---|---|---|
| KCl (mM) | 35 | 0,21 ± 0,01 |
| | 105 | 0,39 ± 0,03*** |
| | 175 | 0,55 ± 0,04*** |
| | 210 | 0,71 ± 0,06*** |
| | 245 | 0,82 ± 0,07*** |
| | 280 | 0,91 ± 0,07*** |
| Adrenaline (µM) | 7 | 0,38 ± 0,06 |
| | 14 | 0,63 ± 0,09 |
| | 28 | 0,96 ± 0,09* |
| | 42 | 0,91 ± 0,10** |
| | 56 | 1,13 ± 0,20*** |
| | 70 | 1 ± 0,05** |

N=5; ** p<0.01, *** p<0.001 Significantly different compared to 7 µM for adrenaline

### 3.3. Effect of prazosin on contractile activity of adrenaline and *P. zenkeri*

Figure 3 and Table 3 represent the effects of prazosin, a specific $\alpha$ adrenergic type 1 inhibitor on adrenaline, *P. zenkeri*-induced deferential contractions. After 30 min of incubation of the vas deferens fragment with 10 M of prazosin, the adrenaline-induced contractions were completely abolished regardless of the adrenaline concentration used. In contrary pretreatment of vas deferens with prazosin induced a partial inhibition of *P. zenkeri* (Fig 3).

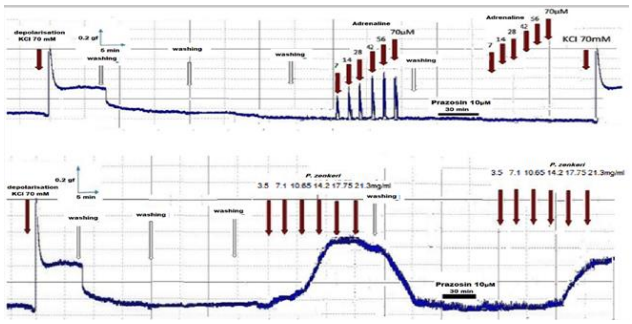


**Figure 3:** Effect of Prazosin on contractile activity of adrenaline and *P. zenkeri*

### 3.4. Effects of Nifedipine on contractile activity of KCl and *P. zenkeri*

Nifedipine, an antagonist of the L-type calcium channels (5 M), administered 30 minutes before KCl, significantly (p<0.001) inhibited the effects of KCl and *P. zenkeri* on the vas deferens (Fig. 4). This inhibition was confirmed by the reduction in the contraction forces obtained at different concentrations (Table 4). With *P. zenkeri*, this inhibition was highest at 21 mg/mL.

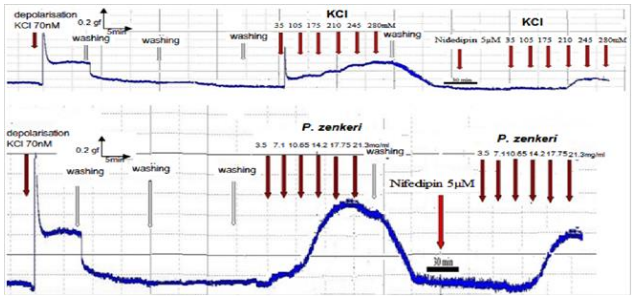


**Figure 4:** Effect of Prazosin on contractile activity of adrenaline and *P. zenkeri*

### 3.5. Effects of adrenaline and *P. zenkeri* on the contractility of vas deferens in a calcium-free medium

In a calcium-free but hyperpotassic medium containing EGTA, the cumulative addition of adrenaline had no effect on the contractile activity of vas deferens (Table V). On the other hand, the contraction recorded at the end of the experiment in the presence of calcium chloride (35 mM) revealed the reactivity of the vas deferens (Fig. 5). Unlike adrenalin, cumulative administration of different concentrations of *P. zenkeri* contracted the vas deferens with remarkable effects at the last three concentrations.

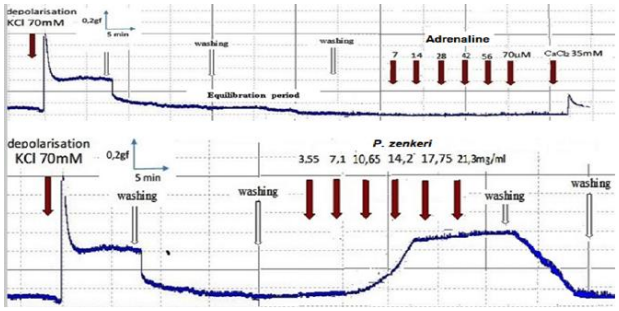


**Figure 5:** Effects of adrenaline and *P. zenkeri* on the contractility of vas deferens in a calcium-free and hyperpotassic medium

### 3.6. Effects of 2-APB on contractile activity of *P. zenkeri* in a calcium-free medium

Fig. 6 and Table 5 present the inhibitory effects of 2-APB (IP3 receptor inhibitor at the sarcoplasmic reticulum) on the contractile activity of aqueous extract of *P. zenkeri* in a calcium-free and hyperpotassic medium. The effects of plant extract were completely inhibited by 2-ABP.

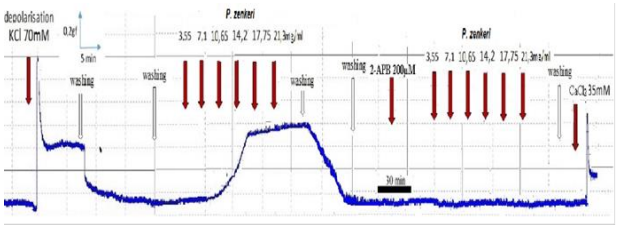


**Figure 6:** Effect of 2-APB on contractile activity of *P. zenkeri* in a calcium-free and hyperpotassic medium

**Table 2:** Effect of prazosin on contractile activity of adrenaline and *P. zenkeri*

| Adrenaline concentrations (µM) | 7 | 14 | 28 | 42 | 56 | 70 |
|---|---|---|---|---|---|---|
| Contraction force of adrenaline in absence of Prazosin (gf) | 0.38 ± 0.06 | 0.63 ± 0.09 | 0.96 ± 0.09*** | 0.91 ± 0.10*** | 1.13 ± 0.20*** | 1.00 ± 0.05*** |
| Contraction force of adrenaline in presence of Prazosin (gf) | 0 | 0 | 0 | 0 | 0 | 0 |
| *P.zenkeri* concentrations (mg/ml) | 3.55 | 7.1 | 10.65 | 14.2 | 17.75 | 21.3 |
| Contraction force of *P.zenkeri* in absence of Prazosin (gf) | 0.19 ± 0.03 | 0.36 ± 0.03 | 0.63 ± 0.03*** | 0.96 ± 0.01*** | 1.27 ± 0.04*** | 1.36 ± 0.04*** |
| Contraction force of *P.zenkeri* in presence of Prazosin (gf) | 0.01 ± 0.01 | 0.04 ± 0.02 | 0.11 ± 0.03 | 0.17 ± 0.04 | 0.22 ± 0.02 | 0.22 ± 0.02 |

N=5; *** p<0.001 Significantly different compared to 7 µM for adrenaline and 3.55mg/ml for *P. zenkeri* respectively

**Table 3:** Effect of Nifedipin on contractile activity of KCl and *P. zenkeri*

| KCl concentrations (mM) | 35 | 105 | 175 | 210 | 245 | 280 |
|---|---|---|---|---|---|---|
| Contraction force of KCl in absence of Nifedipin (gf) | 0.21 ± 0.01 | 0.39 ± 0.03*** | 0.55 ± 0.04*** | 0.71 ± 0.06*** | 0.82 ± 0.07*** | 0.91 ± 0.07*** |
| Contraction force of KCl in presence of Nifedipin (gf) | 0 | 0.03± 0.02 | 0.13± 0.02 | 0.23± 0.01** | 0.30± 0.02*** | 0.36± 0.01*** |
| *P.zenkeri* concentrations (mg/ml) | 3.55 | 7.1 | 10.65 | 14.2 | 17.75 | 21.3 |
| Contraction force of *P.zenkeri* in absence of Nifedipin (gf) | 0.19 ± 0.03 | 0.36 ± 0.03 | 0.63 ± 0.03*** | 0.96 ± 0.01*** | 1.27 ± 0.04*** | 1.36 ± 0.04*** |
| Contraction force of *P.zenkeri* in presence of Nifedipin (gf) | 0.12 ± 0.02 | 0.25 ± 0.03 | 0.47 ± 0.1 | 0.45 ± 0.1 | 0.67 ± 0.1*** | 0.67 ± 0.1*** |

N=5; *** p<0.01, *** p<0.001 Significantly different compared to 35 mM for KCl, N=5; *** p<0.001 Significantly different compared to 3.55 mg/ml for *P. zenkeri*

**Table 4:** Effects of adrenaline and *P. zenkeri* on the contractility of vas deferens in a calcium-free and hyperpotassic medium

| Samples | Concentrations | Mean contraction forces (gf) |
|---|---|---|
| Adrenaline (µM) | 7 | 0 |
| | 28 | 0 |
| | 42 | 0 |
| | 56 | 0 |
| | 70 | 0 |
| *P. zenkeri* (mg/mL) | 3.55 | 0.09±0.01 |
| | 7.1 | 0.2±0.01 |
| | 10.65 | 0.3±0.02 |
| | 14.2 | 0.4±0.02** |
| | 17.75 | 0.5±0.02*** |
| | 21.3 | 0.58±0.02*** |

N=5; ** p<0.01, *** p<0.001 significantly different compared to 3.55 mg/mL

### 3.6. Effects of 2-APB on contractile activity of *P. zenkeri* in a calcium-free medium

Fig. 6 and Table 5 present the inhibitory effects of 2-APB (IP3 receptor inhibitor at the sarcoplasmic reticulum) on the contractile activity of aqueous extract of *P. zenkeri* in a calcium-free and hyperpotassic medium. The effects of plant extract were completely inhibited by 2-ABP.

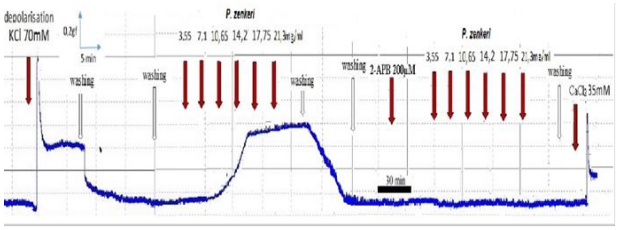


**Figure 6:** Effect of 2-APB on contractile activity of *P. zenkeri* in a calcium-free and hyperpotassic medium

**Table 5:** Effect of 2-APB on contractile activity of *P. zenkeri* in a calcium-free and hyperpotassic medium

| Concentrations of *P. zenkeri* (mg/mL) | Contraction force in absence of 2-APB (200µM) | Contraction force in presence of APB (200µM) |
|---|---|---|
| 3.55 | 0.09 ± 0.01 | 0 |
| 7.10 | 0.2 ± 0.01 | 0 |
| 10.65 | 0.3 ± 0.02 | 0 |
| 14.20 | 0.4 ± 0.02 | 0 |
| 17.75 | 0.5 ± 0.02** | 0 |
| 21.30 | 0.58 ± 0.02*** | 0 |

N=5; ** p<0.01, *** p<0.001 significantly different compared to 3.55 mg/mL

### 4. Discussion

The main objective of this study was to investigate the mechanism of *P zenkeri* on the contractile activity of rat vas deferens with regard to its function in ejaculation. Administration of the aqueous extract of *P. zenkeri* resulted in concentration-dependent contractions of the isolated vas deferens. The contractile effect of KCl is owing to a mechanism related to membrane depolarization and subsequent influx of external calcium through voltage-operated channels [8]. Furthermore, adrenaline receptors are abundant in the proximal part of the vas deferens compared with the distal section. It contracts the vas deferens by interacting with either $a_1$ or $a_2$ receptor [7]. Stimulation of alpha adrenergic receptors leads to the hydrolysis of phosphatidyl inositol into inositol triphosphate ($IP_3$) and diacylglycerol (DAG). $IP_3$ causes the liberation of the intracellular calcium ions, whereas DAG depolarizes the cell membrane inducing calcium ions reflux [9]. The contractile response obtained in this work with the plant extract suggests that its bioactive metabolites mobilize one or more signaling pathways involved in the vas deferens contraction mechanism. The plurality of chemical constituents that characterize plants could also justify the pharmacological effects. Recently, it has been demonstrated that alkaloids and saponins induce contractile activities on smooth muscle [10, 11]. These results obtained with *P. zenkeri* are similar to those obtained by Watcho *et al.* who showed that the aqueous extract of *L. acida* contracted the vas deferens in a dose-dependent manner [12]. This mobilization of calcium to the intracellular medium can be done by various possible signaling pathways. These include adrenergic, purinergic and dopaminergic pathways.

A specific type I adrenergic receptor antagonist, Prazosin was used to explore this signaling pathway in the plant contractile effects. Prazosin at 10 µM competitively blocks alpha-1 adrenergic receptors, thus preventing its agonist from binding [13]. This blockage prevents the increased of intracellular calcium and thereby prevents muscle contraction [13]. From this test, it appears that Prazosin administered in a single concentration inhibited the contractile effects of adrenaline and plant extract. This result suggests that the plant contractile activity is partially mediated the type I adrenergic receptors.

One of the signaling pathways activated via type I adrenergic receptors is the opening of L-type calcium channels [14, 2]. These results are similar to those of [16] who showed that Nifedipine inhibits the effects of norepinephrine. To verify the involvement of L-type calcium channels in the mechanism of plant extract contraction, an inhibition test was carried out using Nifedipine. Nifedipine is an L-type calcium channel antagonist belonging to the dihydropyridine class. It selectively inhibits the entry of calcium ions into the voltage-gated L-type channels [15]. Its administration in the present work partially inhibited both the effects of KCl and those of the aqueous extract of *P. zenkeri*. These results show that the contractile activity of the plant involved the entry Nifedipin inhibits the effects of norepinephrine. Regardless of the agonist (KCl or plant), the use of a single concentration of Nifedipine resulted in decreasing inhibitory capacities with increasing concentration of the agonist. This reflects the competitive nature of the interaction between the two pharmacological substances in each case. This therefore confirms the partial involvement of extracellular calcium via L-type calcium channels in the plant extract mechanism. By removing the extracellular calcium source, more details will be known about the involvement of extracellular calcium in the contractile activity of this plant extract.

The absence of contractile response with adrenaline obtained in calcium free medium confirms the involvement of extracellular calcium in the contraction pathway. In contrast to adrenaline, the exclusion of extracellular calcium did not totally inhibit the effects of the aqueous extract of *P. zenkeri*. This therefore indicates another source of calcium in the contractile activity of this plant. This result could be explained by the fact that the plant extract may mobilize intracellular calcium-to induce the muscle contraction. Therefore, the use of 2-APB allowed to verify this hypothesis.

The 2-APB (IP3 receptor inhibitor) used in the present work inhibited all concentrations of the aqueous extract of *P. zenkeri* showing that the plant extract induced the release of calcium from intracellular reservoirs thus causing muscle contraction. Indeed, the activation of the G protein-coupled receptors also has the consequence of activating the formation

of IP3 whose attachment to its receptors at the level of sarcoplasmic reticulum results in the release of calcium from this intracellular reservoir [10]. Thus, plant extract may directly or indirectly induce calcium release and calcium influx in vas deferent smooth muscles.

**5. Conclusion**

The present results show that *P. zenkeri* is able to induce contractions of the vas deferens and requires the integrity of type 1alpha adrenergic receptors, L-type calcium channels and IP3 receptors. This work confirms the pro-ejaculatory potential of *P. zenkeri* and therefore justifies its use as an aphrodisiac.

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**Statement and declarations**

**Competing interests:** The authors declare no conflict of interest

**Financial interests:** The authors have no relevant financial or non-financial interests to disclose.

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