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Research Article

Pharmacological Effects of MISCA F2 (*Mitracarpus scaber*): A Plant Extract on Intestinal Activity of Rabbit

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Abstract: Plant extract (*Mitracarpus scaber*) coded MISCA is used in traditional medicine to treat most of skin infections. As part of the study of biological tolerance of MISCA, we evaluated the pharmacological effects of the active molecules contained in the ethanol extract of *Mitracarpus sc*aber coded (MISCA F2) on intestinal mechanical activity of rabbit and determine the mechanism of action of these compounds. Our results showed that MISCA F2 dose between 12 µg /ml and 28 µg /ml, causes an increase in rhythmic contractions of rabbit duodenum. This myostimulative action of MISCA F2 is inhibited by atropine at 9.4 $x10^{-10}$ µg / ml and completely stopped in physiological medium poor in calcium ions. These results suggest the presence of cholinomimetic substances in MISCA F2 which favor the entry of calcium into the smooth muscle cells by stimulation of muscarinic receptors. This study may help to establish the scientific basis for biological tolerance of MISCA F2.
Keywords: MISCA F2, Duodenal Contractions, Myostimulation, Myorelaxation, Atropine

INTRODUCTION

Today, all over the world and particularly in Africa, medicinal plants play an important role in healing, in scientific environment as well as in rural and urban areas. This new practice is encouraged especially due to the difficulties faced by western medicine for example lack of medical facilities and high cost of drugs [1-4]. African forest is well endowed in medicinal plants whose effectiveness is well proven. Indeed, more than 5,000 medicinal plant species are listed in the continent [5]. For efficient exploitation of this heritage many research work has be done in other to provide the scientific basis of these plants actions [4-6].

Among many therapeutic plants species, *Mitracarpus scaber* coded MISCA, is among the species mostly used in the treatment of various ailments [7-8]. MISCA is a vegetable substance with wide action on fungal such as *Cryptococcus*, *Candida*, which are inducers of fungal infections. *Candidiasis* caused by Candida, considered to be the first fungal infections in humans and animals, can lead to *oropharyngeal infections*, *gastrointestinal*, leading to invasive forms which are difficult to control [1-2]. MISCA also has an effect on the *Trichophytons*, the *Microsporum*, *Aspergillus* and *salmonella* and mycobacteria such as *Mycobacterium ulcerans*, the causative agent of Burili ulcer [9-11]. Clinical trials of the effectiveness of MISCA in humans were made through the development of MISCA pharmaceutical creams, healing with success, superficial and skin mycoses [12-10].

As part of the study of biological tolerance of MISCA, we evaluated the pharmacological effects of the active compound contained in the ethanol extract of *Mitracarpus scaber* (MISCA F2) on intestinal mechanical activity of rabbit and determine the mechanism of action of these compounds.

MATERIALS AND METHODS

Plant Material

The plant material used is a powder obtained from the aerial parts (leaves, stems and flowers) of *Mitracarpus scaber* (Rubiaceae) [13-15]. This plant has been identified by an expert of the National Centre of Floristic Côte d' Ivoire, where samples of this plant have been kept.

Animals

The rabbits of the genus *Oryctolagus cumiculu*s (Leporidae) weighing 2 to 2.5 kg were used for the study of intestinal contractility. They were brought from farms in the suburbs area of Abidjan (Côte d'Ivoire). They are acclimated in the pet unit of the Training and Research Unit (UFR) of the faculty of Biosciences, University of Cocody for a week, to regulate and harmonize their physiological state before the experiments. In pet unit, the average temperature 26 ± 4°C with a relative humidity of 60% and a photoperiod of 12 /24 were maintained. Animals were fed with ad libitum pellets and were given water.

Chemical

The chemical substance used is Atropine (Atr.) [Prolabo, France]. This is a competitive inhibitor of

acetylcholine (ACh) and muscarinic cholinergic agonists.

Physiological Solution

The saline solution used was according to Mac Ewen, 1956 [16]. It is composed of NaCl (130), KCl (2.5), $CaCl_2$ (2.42), Na_2HPO_4 (1.18), CO_3HNa (11.9), $MgCl_2$ (0.24), glucose (2.2) in (mM). This solution was used at a pH of = 7.4.

Preparation of the plant extract

The dry powder (leaves, stems and roots) of *Mitracarpus scaber* coded MISCA is extracted with ethanol (100 g /l) using a magnetic stirrer (IKAMAGRCT) for 24 hours, the extract obtained was filtered through a Buchner funnel with WATTMAN (3 mm) paper and evaporated at reduced pressure to 30°C using a rotary evaporator ROTAVAPOR BÜCHI. We obtain by this method, the total ethanolic extract undergoing column chromatography 3X10 cm Sephadex G2. This chromatographic fraction is evaporated at reduced pressure to temperature of 30°C using a rotary evaporator Buchi Rotavapor, which gives the fraction MISCA F2.

Experimental settings and technical recording of the mechanical activity of isolated intestine of rabbit

The experimental apparatus includes a water bath whose temperature is controlled by a thermostat. In this bath immersed a vat for containing isolated intestine. The latter is supplied with physiological solutions of the type Mac Ewen, from bottles placed 40 cm above the apparatus. Before arriving at the tank containing the organ, the liquid contained in the bottles pass through polyvinyl catheters and coils that allow these solutions to heat up and to maintain the same temperature as the water bath at 38°C. The liquid supply is controlled by a multi- tap selection channels. The isolated organ vessel is emptied through a vent at the bottom of the apparatus.

The rabbit was fasted for 24 hours. After the preparation of the various solutions and experimental settings, it was sacrificed. After a midline laparotomy, segments of duodenum 3 cm long, was immediately removed and kept alive in a Mac Ewen solution of glucose and oxygenated. By means of a thread passed through the wall of the duodenum, a node was done at one end of the fragment to hang the isolated organ inside the vessel. The other end was connected by another thread, to the end of the stylus in contact with a smoky cylinder rotated at constant speed. The test substances were diluted in a Mac Ewen solution and introduced into the isolated organ vessel with a graduated syringe.

Record

Recordings made on smoked paper were painted to fix the black smoke, and then scanned before being reversed using photo editor and paint software by Microsoft. The curves were plotted using the Graph Pad Prism 4 software (Microsoft, San Diegi, California, USA).

Statistical Analysis

Statistical data are expressed as mean ± standard error (M ± SEM) obtained from (n) separate experiments.

RESULTS

Dose-dependent action of MISCA F2 on the contractile activity of the duodenum

MISCA F2 concentrations (12 to 28 µg/ml) causes progressive increase in duodenal contractile activity (100 to 200 %) corresponding to the maximum amplitude generated by the dose of 28 µg / ml (Figure 1). The graph in Figure 2, which shows the increase in duodenal contractile activity depending on the dose of MISCA F2 was used to determined the effective dose (ED_{50}) equal to 19µg /ml.

Dose-dependent action of Atr on the contractile activity of the duodenum

Increasing doses of atropine (4 to 20^{-10} µg /ml) induced relaxation of duodenal contractions (100 to 20%) with an ED_{50} equal to 9.4 $x10^{-10}$ µg / ml (Figures 3 and 4).

Antagonistic action of MISCA F2 on duodenum contractions initiated by atropine

The ED_{50} of atropine (9.4 $x10^{-10}$ µg / ml) was added to the bath containing duodenal tissue and immediately after it's action it was added to the same bath MISCA F2 (19 mg / ml). The myostimulating action of MISCA F2 was inhibited by that of atropine (Figure 5).

Action of MISCA F2 in a hypocalcic and hypercalcic medium

The ED_{50} dose of MISCA F2 (19 µg /ml) was injected into the tissue bath of a physiological solution poor in calcium (25%), duodenal contractions was reduced from 175 to 100%. As against when injecting ED_{50} of MISCA F2 in the calcium-enriched saline (150%), duodenal contractions increased from 100 to 200 % (Figure 6).

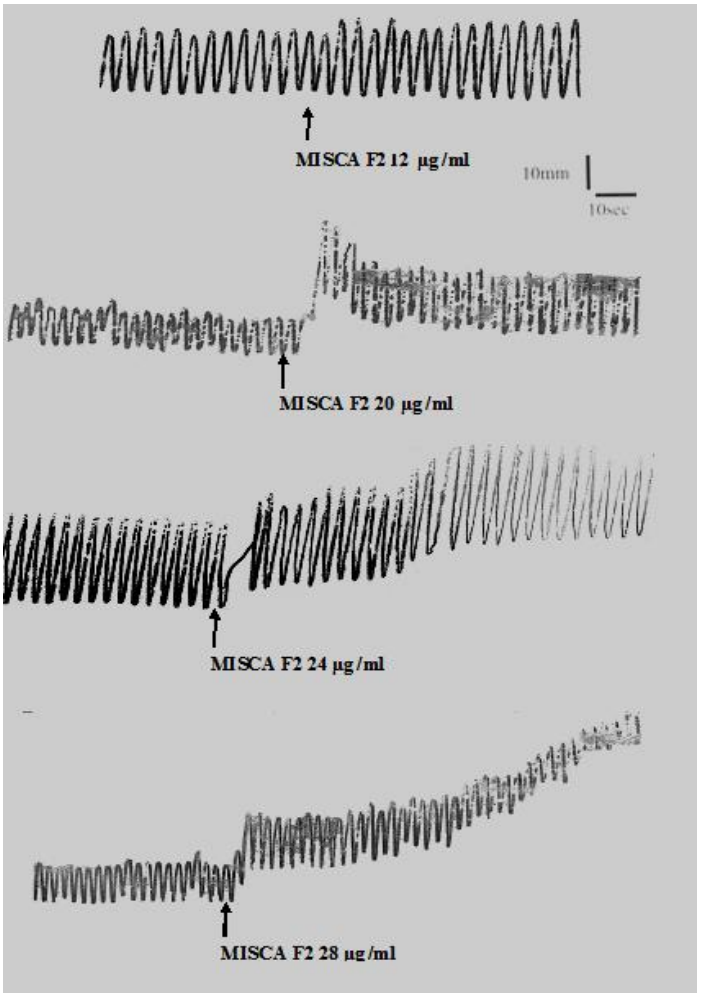

Fig.1: Influence of increasing- dose of MISCA F2 on contraction of rabbit duodenum

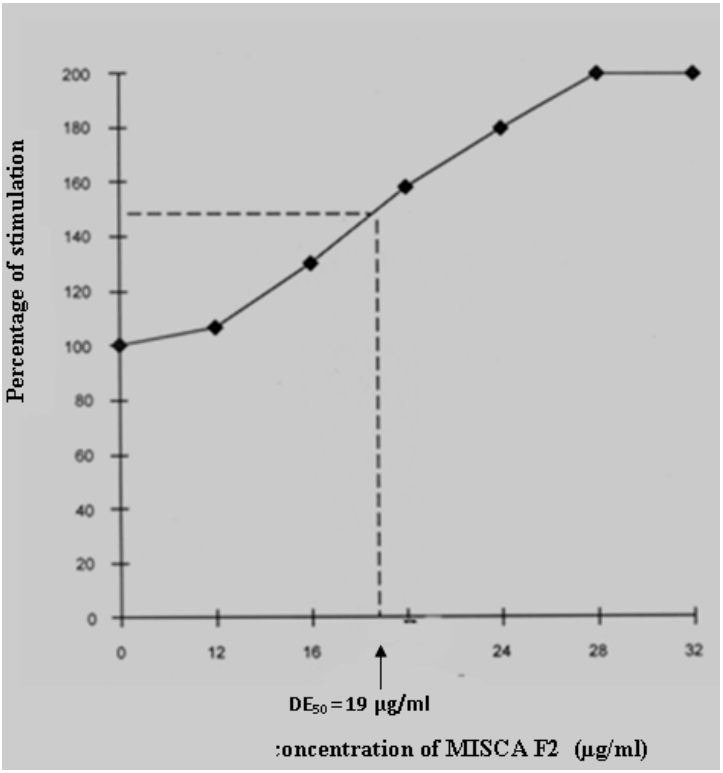

Fig. 2: Action of increasing dose of MISCA F2 on contractions of rabbit duodenum

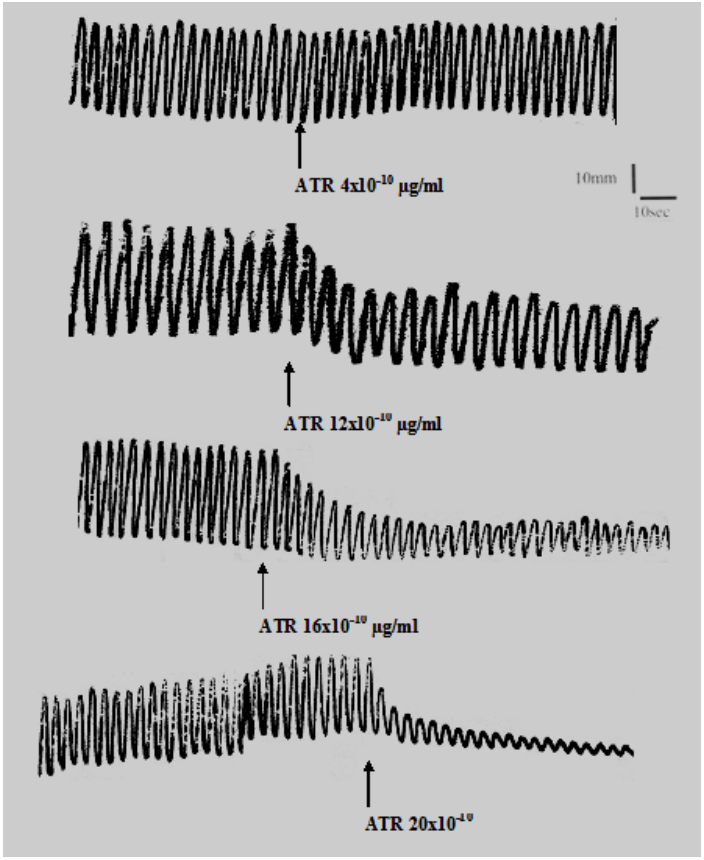

Fig. 3: Influence of increasing dose of Atr on the contraction of rabbit duodenum

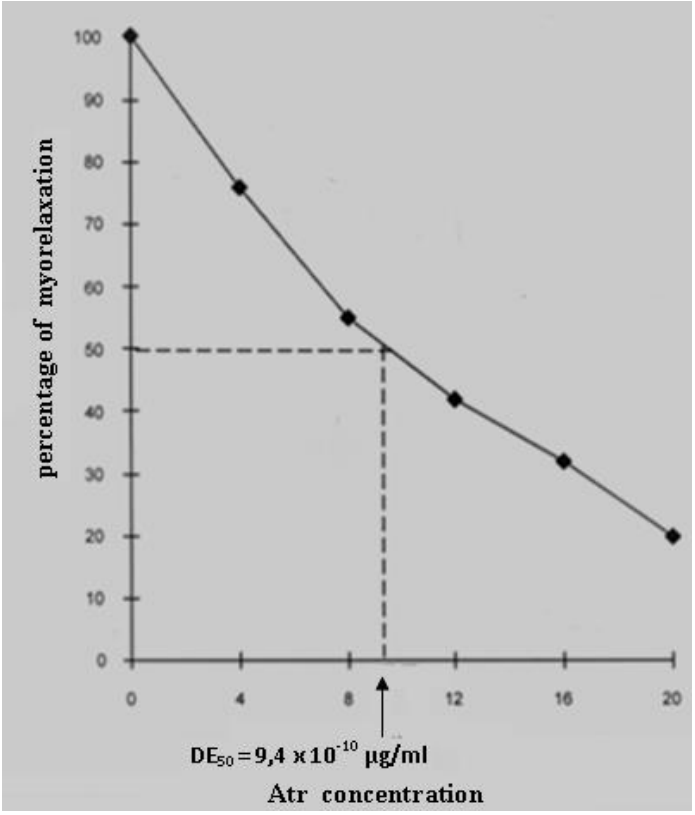

Fig. 4: Action of increasing dose of Atr on the contraction of rabbit duodenum

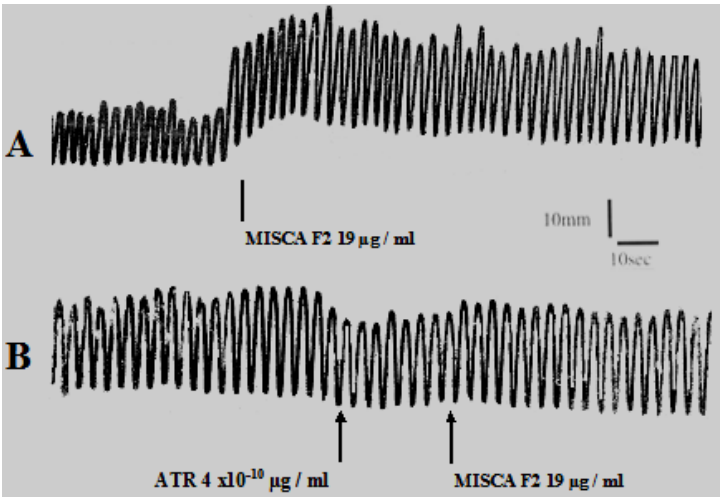

Fig. 5: Action of MISCA F2 on the contraction of rabbit duodenum based on time ,in the absence (A) and in the presence of (B) Atr

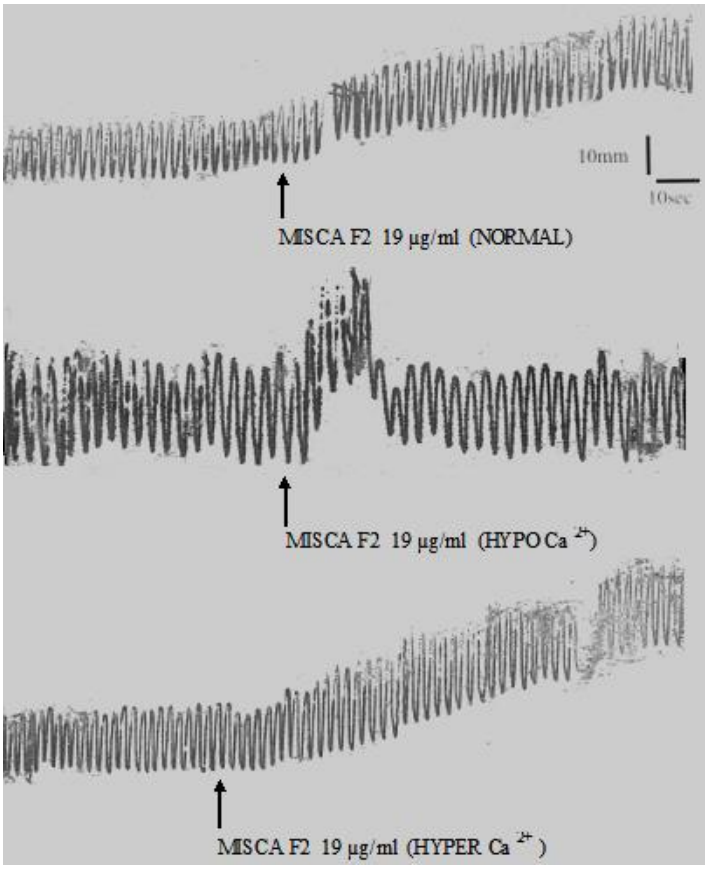

Fig. 6: Influence of calcium on the contraction of duodenum induced by MISCA F2

DISCUSSION

Our concern in this study was to evaluate the pharmacological effects of the active compounds contained in the ethanol extract of *Mitracarpus scaber* on intestinal mechanical activity of rabbit and determine the mechanism of action of these compounds.

MISCA F2 between 12 µg / ml and 28 µg / ml induced a dose dependent increase in the amplitude of

rhythmical contractions of rabbit duodenum. The 50% effective dose (ED_{50}) is equal to 19µg / ml.

The physiological effect of MISCA F2 on contractions of rabbit duodenum is comparable to aqueous extracts of *Swartzia madagarensis* and *Mareeya micrantha* on intestinal contractile activity of rabbit [17-18] and aqueous extracts of *Khaya senegalensis*, of *Citrus aurantifolia* and on the smooth muscle of *Taenia coli* [19]. The effects of MISCA F2 on contractions of rabbit duodenum are similar to those of acetylcholine on smooth muscle visceral [20].

The study of antagonism Atropine-MISCAF2 indicates that the increase in rhythmic contractions of rabbit duodenum induced by MISCA F2 at 19 µg/ml was completely eradicated by atropine at 9.4 $x10^{-10}$ µg / ml. This experiment showed that MISCA F2 contains muscarinic cholinergic substances responsible for the increase in rhythmic contractions of rabbit duodenum. Muscarinic receptors are coupled to G proteins, the adenyl cyclase, ions channel that cause increase in calcium [20-21]. Therefore, they may well encourage the entry of calcium into the smooth muscle cell and cause the release of calcium from intracellular reserves responsible for myostimulating action of MISCA F2 on contractions of rabbit duodenum.

Therefore, we studied the effects of MISCA F2 at 19 µg / ml on the contractile activity of rabbit duodenum in hypocalcic and hypercalcic physiological medium. In hypocalcic physiological environment, myostimulating action of MISCA F2 at 19 µg/ ml was inhibited while this action was well pronounced in hypercalcic environment. These results showed the importance of calcium ions in myostimulating action of MISCA F2. In hypocalcic medium MISCA F2 have muscle relaxant effects. These results suggest that cholinergic substances in the ethanolic extract of *Mitracarpus scaber*, like acetylcholine causes the elevation of rhythmic contractions of the rabbit duodenum by encouraging the entry and release of calcium into the smooth muscle cell by activation of the muscarinic receptors [20-22]. The entry of calcium, source of calcium ions into the visceral smooth muscle may cause secondary release of intracellular calcium [21]. If our results showed the presence of cholinomimetic substances in MISCA F2, muscle relaxant effects induced by MISCA F2 in the presence of atropine and physiological medium poor in calcium, suggest the presence of other substances other than cholinergic substances in the ethanolic extract of *Mitracarpus scaber.*

CONCLUSION

The study of the effects of MISCA F2 on the rhythmic activity of rabbit duodenum revealed that MISCA F2 dose between 12 µg / ml and 28 µg / ml, causes a dose dependent increase of rhythmic contractions of the smooth muscle of rabbit duodenum. This myostimulating action of MISCA F2 appears to be related to the presence of muscarinic cholinomimetic substances. These substances favor the entry of calcium into the smooth muscle cells by activation of muscarinic receptors.

This study may help to establish the scientific basis of biological tolerance of MISCA F2. Future experiments could be used to isolate and characterize the active molecules present in the MISCA F2 fraction.

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