Original Research Article

Effects of Fractions of MISCA (*Mitracarpus scaber*), on Contractions of Rabbit Duodenum

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ABSTRACT

The plant extract (*Mitracarpus scaber*) coded MISCA is used in traditional medicine to treat most of the skin diseases. As part of the study of biological tolerance of MISCA, we were required to determine the physiological properties of the three extracts of MISCA (total ethanolic extract, F1 and F2 fractions) on the contractions of isolated duodenum of rabbit. The two fractions of MISCA were separated by the method of column chromatography 3x10 cm with Sephadex G10. These extracts were tested at concentrations ranging from 120 to 480 µg / ml on spontaneous contractions of isolated duodenum of rabbit. The results showed that the total ethanolic extract and F2 fraction of MISCA, cause myostimulation contractions ranging from 100 to 160%, and 100 to 280%, respectively. F1 fraction has two types of action, myostimulation from 100 to 166% followed, after a few seconds, by muscle relaxation from 160 to 30%. This study may help to establish the scientific basis for biological tolerance of MISCA on the rabbit duodenum.

Introduction

Today, all over the world and particularly in Africa, medicinal plants play an important role in therapy in rural and urban areas as well as in scientific environment. This new practice is encouraged due to the difficulties faced by western medicine for example lack of health infrastructure and the high cost of drugs (Kporou et al., 2009, 2010, Okou et al., 2008, Yapi et al., 2011). The African floristic is very rich in medicinal plants whose effectiveness is proven, in fact, more than 5,000 medicinal plants species is found in the continent (Adjanohouan et al., 1979). For the efficient exploitation of this African heritage many research has
been done to provide scientific bases of these plants actions (Yapi et al., 2011, Dieng et al., 2005). Among many plants species used for their therapeutic effect, Mitracarpus scaber coded MISCA, is among the most used in the treatment of various ailments (Okou et al., 2005, Zihiri et al., 2007). MISCA is a vegetable substance with wide range effect on fungal organisms such as Cryptococcus, Candida, which are inducers of fungal infections. Candidiasis caused by Candida, a major fungal infection in humans and animals, can cause oropharyngeal infections and gastrointestinal infections (Kporou et al., 2009, 2010). MISCA also has an effective action on the Trichophytons, Microsporum, Aspergillus and salmonella and on mycobacteria such as Mycobacterium ulcerans, the causative agent of ulcer Burili (Thes et al., 2005, 2011, Wels et al., 2006). Clinical trials on the effectiveness of MISCA were made by the production of pharmaceutical creams MISCA used on humans to treat successfully superficial and cutaneous mycoses (Guede-Guina et al., 1996, 1998, and Thes et al., 2011). As part of our study on biological tolerance of MISCA, we were required to determine the physiological effect of the three extracts of MISCA (total ethanolic extract, F1 and F2 fractions) on the contractions of isolated duodenum of rabbit.

Materials and Methods

Plant Material

The plant material is the powder made from the aerial parts (leaves, stems and flowers) of Mitracarpus scaber (rubiacae) plant (De Souza et al., 1997, Toudji et al., 1997 and Trebissou et al., 2002). 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.

Animal Material

Rabbits of the genus Oryctolagus cuniculus (Leporidae) weighing 2 to 2.5 kg were used for the study of intestinal contractility. They were brought from farms in the suburb of Abidjan (Côte d'Ivoire). They were acclimated in the pet Unit of the Faculty of (UFR) Biosciences, University of Cocody for a week, to regulate and harmonize their physiological state before the experiments. In this pet site, the average temperature is 26 ± 4 °C with a relative humidity of 60% and a photoperiod of 12 /24. These animals are fed with ad libitum and water.

Reference physiological Solution

Reference physiological solution used was Mac Ewen, 1956. It is composed in (mM) of NaCl (130), KCl (2.5), CaCl2 (2.42 ), Na2HPO4 (1.18 ) CO3HNa (11.9), MgCl 2 (0.24), glucose (2.2). This solution is used at a pH = 7.4.

Preparation of plant extracts

The dry powder of Mitracarpus scaber (rubiacae) coded MISCA was extracted with ethanol (20g/l) subjected to magnetic stirring for 24 hours. This extract was filtered through Whatman 3 mm paper and evaporated at 30 ° C under a reduced pressure in a Rotavapor Buchi type. The total ethanol extract obtained undergoes a chromatography in column 3x10 cm with Sephadex G10. The eluent with 20 % ethanol gives F1 fraction while the one made with 100 % ethanol gives the fraction F2 (Trebissou, 2001).
Experimental apparatus and recording of the mechanical activity of isolated intestine of rabbit

The experimental apparatus includes a water bath whose temperature is controlled by a thermostat. Immersed in the water bath is an isolated organ vessel, for containing the piece of intestine. The latter is maintained in physiological solutions Mac Ewen type, from bottles placed 40 cm above the apparatus. Before getting into the vessel, the liquid contained in the bottles go through polyvinyl catheters and coils that allow these solutions to warm and to maintain the same temperature as that of 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 goes through fasting for 24 hours. After the preparation of the various solutions and experimental device, it was sacrificed. Following a midline laparotomy, segments of duodenum 3cm long was immediately removed and kept alive in a Mac Ewen solution of glucose and oxygen. By means of a thread passed through the wall of the duodenum, a node was made at one end of the fragment to allow it to hang inside the isolated organ vessel.

The other end was connected by another thread; the end of the stylus is in contact with a smoky cylinder rotated at constant speed. The test substances are diluted in a solution of Mac Ewen and brought to the isolated organ vessel through a graduated syringe.

The preparation was subjected to the direct action of different concentrations of MISCA ranging from 120 to 480 micrograms / ml.

Analysis and recording

Recordings made on smoked paper are painted to fix the black smoke, and then scanned before being retouched using software photo-editor and Paint by MICROSOFT. The curves were plotted using 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.

Result and Discussion

Dose-response effect of MISCA (total extract) on the rhythmic contractions of rabbit duodenum.

Figure 1 shows the effects of total ethanolic extract of MISCA in the rabbit duodenum based on concentration. Before the MISCA action, the amplitude of spontaneous contractions is normal (100%). From 120 to 480 µg / ml of MISCA, there was an increase in the amplitude of rhythmic contractions from 100 to 166%. The mean values obtained after several experiments (n = 5) reflect the same phenomenon (Figure 4).

Dose-response effect of fraction F1 of MISCA on rhythmic contractions of rabbit duodenum.

Figure 2 shows the effects of MISCA F1 on rabbit duodenum based on concentration. Up to 120 µg / ml, normal rhythmic contractions of the rabbit duodenum was recorded. From 120 to 480 µg / ml of MISCA F1, there was an increase in the amplitude of contractions,
increased from 100 to 145%, followed by an important drop, after a few seconds, of the amplitude of contractions (145 to 30%) between 240 and 480 µg / ml of MISCA F1 (Fig. 4).

**Dose-response effects MISCA F2 on rhythmic contractions of rabbit duodenum.**

MISCA F2 of (120 to 480 µg / ml) concentration caused progressive increase in duodenal contractile activity from 100 to 280% (Figs 3 and 4).

Our job was to, in the context of the study of biological tolerance of MISCA, determine the physiological basis of the three extracts of MISCA (total ethanolic extract, F1 and F2) on the contractions of isolated rabbit duodenum. In the African pharmacopoeia, this plant is used to treat most of the skin diseases (Kra et al., 2002, Vangah-Manda et al., 1993, Germano et al., 2005 and Kporou et al., 2009).

Our results showed that the total ethanolic extract and F2 fraction of MISCA, concentrations from 120 to 480 µg / ml, induced myostimulations duodenal contractions of rabbit from 100 to 166% and 280 % respectively. As against the F1 fraction of MISCA which has two types of action, myostimulation contractions at concentrations of 120 to 240 µg / ml which increased from 100 to 145% then in a few seconds followed by a myorelaxation at concentrations (360 to 480 µg / ml) of 145 to 30%.

It has been shown that any substance like acetylcholine (ACh), has a cholinomimetic action raises the amplitude of gastrointestinal movements can be classified as diarrheal substance and stimulates contraction of tissue muscle. It is myostimulate. On the contrary plant substances that reduce gastrointestinal contractions such as atropine (ATR) are called anti-diarrheal and are muscle relaxant (Bahi, 2000).

The myostimulation duodenal contractions have been reported. Indeed in 1997, the action of *Mareeya micrantha*, an *Euphorbiaceae* used by peoples for its laxative properties. The action of this plant on duodenal contractions of rabbit resulting in stimulation of rhythmic contractions (Guede-Guina et al., 1997, 1991 and Dosso, 2000). In general, laxative substances induce a myostimulation contraction of rabbits, duodenum through their agonistic effects on cholinergic receptors. These plants have an action similar to that of Ach, a reference mobility mediator, they are said to be cholinomimetics (Trebissou et al., 2002 and Bahi et al., 1998).

MISCA F1 behavior on duodenal contractions of rabbit has been reported by several researches. In 1981, the study on *Morinda morindoides* coded (Bitter GG), a *morindacée* of Côte d' Ivoire, the effect of this plant on duodenal contractions revealed that this plant induces a myostimulation followed by a duodenal muscle myorelaxation (Bahi et al, 1998). These results were reported in 2008, testing the effects of TOBACOAK, a plant extract used in the treatment of AIDS. The effect of the alcoholic extract of TOBACOAK on duodenal tissue showed that at lower concentrations (120 and 360µg/ml) a myostimulation, followed by a myorelaxation of duodenal contractions at high concentrations (360-760 mg / ml ) (Okou et al, 2008). The same results were reported in 2009 by research done on the
Figure 1 Dose-dependent influence of the total extract of MISCA on contractility of rabbit duodenum

MISCA (Ext. T) 120 µg.ml⁻¹

MISCA (Ext. T) 240 µg.ml⁻¹

MISCA (Ext. T) 360 µg.ml⁻¹

MISCA (Ext. T) 480 µg.ml⁻¹
Figure 2 Dose-dependent influence of F1 fraction of MISCA on the contractility of rabbit duodenum
Figure 3: Dose-dependent influence of F2 fraction of MISCA on the contractility of rabbit duodenum.

- MISCA F2 120 µg.ml⁻¹
- MISCA F2 240 µg.ml⁻¹
- MISCA F2 360 µg.ml⁻¹
- MISCA F2 480 µg.ml⁻¹
Figure 4: Dose-dependent influence of F2 fraction of MISCA on the contractility of rabbit duodenum.

Stimulation percentage

![Graph showing dose-dependent influence of F2 fraction of MISCA on contractility of rabbit duodenum.]

Concentration of MISCA in µg/ml

pharmacological effects of aqueous extract of Mirabilis jalapa L. (Nyctaginaceae) (Kouadio et al., 2009 and Nene Bi et al., 2009).

These results suggest that (Mitracarpus scaber) F1 fraction of MISCA may contain two types of compounds, each responsible for transient myostimulation duodenal contractions while the other would be responsible for myorelaxation of duodenal contractions.

This study may help to establish the scientific basis for biological tolerance of MISCA. Future study could investigate by isolating and characterizing the active molecules present in the different extracts of MISCA.

References


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