
# Asian Journal of Biochemistry

ISSN 1815-9923

Academic
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Asian Journal of Biochemistry 8 (1): 24-32, 2013
ISSN 1815-9923 / DOI: 10.3923/ajb.2013.24.32
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# Cholinergic and Histaminergic Activities of the Aqueous Extract of *Mareya micrantha* (Benth). Müll Arg (Euphorbiaceae)

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## ABSTRACT

*Mareya micrantha* (Benth). Müll Arg (Euphorbiaceae) is a medicinal plant used for its laxative, abortive and antibacterial properties. This work aims to study the mechanism of the stimulant effects of the aqueous extract of *Mareya micrantha* on rabbit duodenal contractility. Rabbits were sacrificed and the duodenum was isolated. Increasing doses of the aqueous extract were antagonized with 1,1-dimethyl-4-diphenylacetoxypiperidinium iodide (M3 muscarinic receptor antagonists) and fexofenadine (H1 histaminergic receptor antagonist) on rabbit duodenum contractions. *Mareya micrantha* (40-240 μg $mL^{-1}$) induced dose-dependant contractions inhibited by 1,1-dimethyl-4-diphenylacetoxypiperidinium iodide ($10^{-8}$ M) and fexofenadine ($3.17\ 10^{-7}$ M), indicating an action *via* type M3 muscarinic and type H1 histaminergic receptors. The aqueous leaf extract of *Mareya micrantha* contains saponins, alkaloids, polyphenols which are probably responsible for the observed results. The $LD_{50}$ (566.66 mg $kg^{-1}$ of body weight) indicated that the extract has a low toxicity according to toxic substances classification. These results show that this extract contains M3 cholinergic receptor and H1 histaminergic receptor agonist. The contractile activity of the aqueous extract of *Mareya micrantha* shown in our study can justify the use of this plant as a laxative in traditional medicine.

**Key words:** *Mareya micrantha*, fexofenadine, 1,1-dimethyl-4-diphenylacetoxypiperidinium iodide (4-DAMP), laxative properties, antibacterial extracts

## INTRODUCTION

Different classes of chemical drugs such as laxatives are used in the conventional medicine to heal constipation (Najeeb-ur-Rehman *et al*., 2012), gut motility disorder which is considered as a root cause of ill health (Mehmood *et al*., 2011). Herbal remedies are relatively safe and a single plant can offer multiple therapeutic benefits (Gilani and Atta-ur-Rahman, 2005).

*Mareya micrantha* (Euphorbiaceae) commonly known as "Oyia" in Attié, a local language of Côte d'Ivoire (West Africa), is a plant widely used in traditional medicine in West and Central Africa for the treatment of constipation (Meite *et al*., 2010) and many diseases which require drastic actions such as Tapeworm infections, gonorrhea and leprosy (Macfoy and Cline, 1990). Leaves and stems barks of *Mareya micrantha* have been identified to possess the potency to cure trypanosomiasis and leishmaniasis (Djaman *et al*., 2011). The combination of *Mitracarpus scaber*,

*Cassia alata* and *Mareya micrantha* provided a total cure for ringworm (Thes *et al.*, 2011). In Ghana, leaves of *Mareya micrantha* are boiled together with cut fruits of *Citrus sinesis* and decoctions drunk for the treatment of malaria (Asase *et al.*, 2012). In the South of Côte d'Ivoire, *Mareya micrantha* leaves are currently used as laxative and also as oxytocic agent (Guede-Guina *et al.*, 1995; Tsai *et al.*, 1995).

The phytochemical studies on *Mareya micrantha* extracts revealed the presence of chemical groups, such as, flavonoids, alkaloids, tannins, polyphenols, sterols and polyterpenes (Meite *et al.*, 2010). *Mareya micrantha* extract has also been evaluated pharmacologically and shown to possess antibacterial activities (Macfoy and Cline, 1990). Furthermore, the aqueous leaf extract has been reported to show gut stimulatory activity via activation of muscarinic and other receptors (Guede-Guina *et al.*, 1995). On the other hand, the aqueous leaf extract of *M. micrantha* suppressed cardiac contractility of isolated frog and rat hearts in a concentration dependent way (Guede-Guina *et al.*, 1995).

The laxative effects of the aqueous extract of *Mareya micrantha* were studied in rats (Meite *et al.*, 2010). In a previous study, the stimulant effects of this extract have also been shown in guinea-pig ileum (Tsai *et al.*, 1995). These authors had shown that *Mareya micrantha* possesses stimulant effects due in part to an activation of muscarinic receptors (Meite *et al.*, 2010). However, at this time, there are no studies indicating the type of muscarinic receptor and the nature of the other receptors involved in the genesis of these effects. The objective of the present work was to study the role played by type M3 muscarinic receptors and H1 histaminergic receptors in the stimulant effect of *Mareya micrantha* on duodenal muscles of rabbit.

## MATERIALS AND METHODS

**Animal:** Rabbits (1.5-2 kg) and Swiss albino mice of both sexes (18-30 g) were housed and bred in the animal house of UFR-Biosciences at Cocody University in Abidjan (Côte d'Ivoire). They were kept at 25-30°C with free access to food and water. 24 hours prior to the experiments, the rabbits were deprived of food.

**Plant material:** The fresh leaves were collected from *Mareya micrantha* plants located at Akoupé, in June 2007, identified and authenticated by Pr AKE ASSI at the department of botany, University of Cocody. After identification, a voucher specimen (N°1804) was deposited in the herbarium of "Centre National de Floristique" of the University of Cocody-Abidjan.

### Methods

**Extraction procedure:** The leaves of *M. micrantha* were air-dried and then crushed. Eighty grams of the dry powder were mixed with two liters of distilled water. The mixture was shaken for 24 h, at room temperature by means of an agitator (AGIMATIC-N). The macerated mixture was filtered and the filtrate was evaporated in a carefully regulated water bath (maintained at temperature of 70°C) to yield a dark solid extract. According to the dose, the dried extract was then solubilized in distilled water and was used as the aqueous extract of *Mareya micrantha* (MAR) during physiological and toxicological investigations.

**Isolated rabbit duodenum preparation:** The spasmogenic activities of the plant material were studied using isolated rabbit duodenum preparations as described by Tsai *et al.* (1995). Rabbits were sacrificed by cervical dislocation. Segments of 3 cm length of rabbit duodenum were quickly

dissected and cleaned of connective tissues. The isolated rabbit duodenum was removed and set up for recording isometric contractions in organ bath containing Mac Ewen solution (250 mL), at 38°C with oxygen. The isolated rabbit duodenum was mounted in organ bath by suspending it between two stainless steel hooks vertically.

**Determination of the pharmacologic mechanism of action of MAR:** The effect of various concentrations of MAR was tested. The extract was added to the solution in the organ bath and the contractions of the duodenum were recorded. Between applications of MAR, the solution in the organ bath was replaced and the tissue was allowed to rest for 5-10 min. In the second part of the test, antagonists were added 3-5 min prior testing the action of MAR and the contractions were recorded. The 50% effective concentrations (EC50) were determined in the two cases and compared.

**Phytochemical screening:** The aqueous extract of *Mareya micrantha* was subjected to phytochemical screening for the presence of alkaloids, flavonoids, tannins, sterols, terpenoids, phenolic compounds, saponins, using standard procedures of analyze (Silva *et al*., 1998).

**Acute toxicity:** Male and female S*wiss albino* mice weighing 18-30 g were used and they were randomly divided in groups with ten mice in each group. Each group of mice was placed in the cage for a 30 min habituation period before any intra-peritoneal injection of the drug. First group of mice was injected a saline solution as negative control. The test used increasing doses (400-800 mg $kg^{-1}$) of the plant (MAR) extract injected to the other groups of mice serving as test groups. Each mouse in a group received the same dose of MAR through intra-peritoneal (i.p.) injection. The mice were nourished for 24 h and kept under regular observation in order to check mortality and behavioral changes. $LD_{50}$ was determined using both graphical and calculation methods (Nene Bi *et al*., 2008).

**Data analysis:** To normalize the result for the different duodenum segments mentioned above, the contraction in response to MAR was expressed as a percentage of the maximal response to MAR obtained for each tissue. A statistical comparison of the mean responses in the presence of the various inhibitors was performed using a paired t test. The $p<0.05$ was considered statistically significant. Data analysis has been performed using Graph pad prism 4 (Graph pad, San Diego, CA, USA).

## RESULTS

**Phytochemical screening:** Phytochemical investigations carried out on *Mareya micranhta* revealed the presence of many active constituents such as phenolic compounds, alkaloids, flavonoids, saponosids, terpenoids, sterols and tannins.

**Acute toxicity:** The aqueous extract at 400 mg $kg^{-1}$ produced no mortality in mice. Higher doses (500-800 mg $kg^{-1}$) produced dose-dependent mortality and the $LD_{50}$ were 562.34 mg $kg^{-1}$ b.wt. (method graphic); 566.66 mg $kg^{-1}$ b.wt. in mice (calculated) (Fig. 1).

**Effect of MAR on isolated rabbit duodenum preparation:** As shown in Fig. 2, MAR at 40 to 240 μg $mL^{-1}$ exerted a dose-dependent-spasmogenic effect on duodenal smooth muscle isolated from rabbit with an 50% effective concentration (EC50) value of 133.33±45 μg $mL^{-1}$. The records obtained at 40 μg $mL^{-1}$ and 80 μg $mL^{-1}$ showed a no significant ($p>0.05$) increase in the amplitude

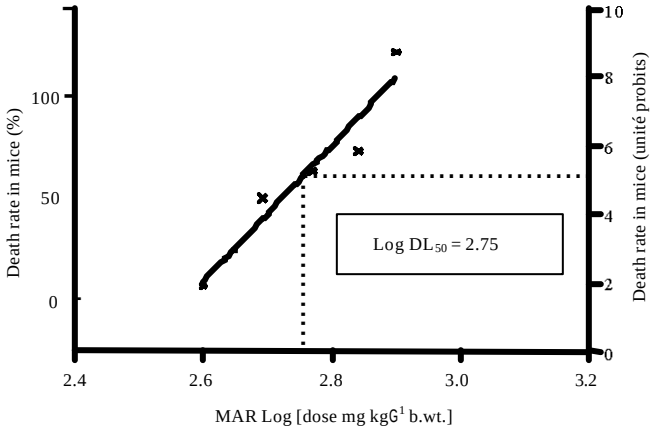


Fig. 1: Trends in mortality according to dosages of *Mareya micrantha* in mice, Log $LD_{50}$ = 2.75 ($LD_{50}$ = 562. 34 mg $kg^{-1}$ b.wt.) The animals were treated by intraperitoeal (i.p) administration of MAR

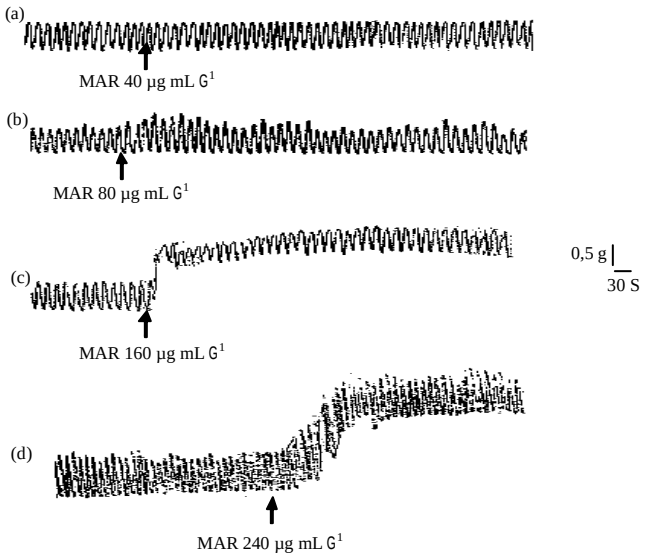


Fig. 2(a-d): Dose-response effect of *M. micrantha* on rabbit duodenum

of the spontaneous contraction. At 160 µg $mL^{-1}$ of MAR, the increase (1281±45 mg) in the amplitude of duodenal contraction was significant ($p<0.01$), while it was more (1833.42±45 mg) significant ($p<0.001$) at the dose of 240 µg $mL^{-1}$ of the extract.

**Effects of fexofenadine (FEXO) on contractions elicited by the aqueous extract of *M. micrantha*:** In the rabbit duodenum, the aqueous extract of MAR produced a dose-dependent stimulatory effect at 80-240 µg $mL^{-1}$, which was significantly blocked in the presence of FEXO at the dose $3.1710^{-7}$ M (Fig. 3), with an EC50 value of 205±3.87 µg $mL^{-1}$ (Fig. 4). The duodenum contraction induced by MAR at 80 µg $mL^{-1}$ was a no significant decrease by FEXO ($p>0.05$). However, the increase of the contractile force induced by MAR (160-240) was reduced from 58±1.9 to 42±2.5%, in the presence of FEXO ($p<0.01$-$0.001$).

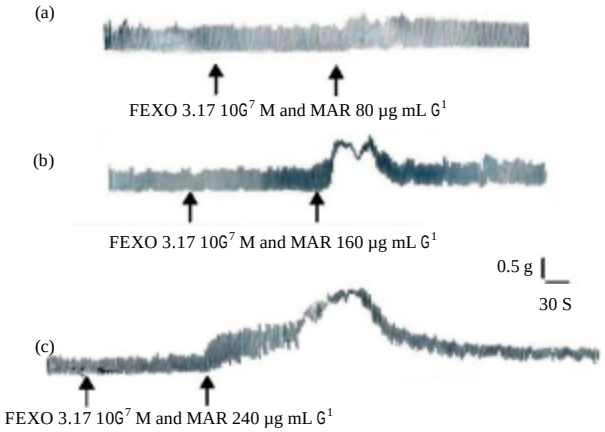


Fig. 3(a-c): Interation effect between FEXO and MAR on rabbit duodenum

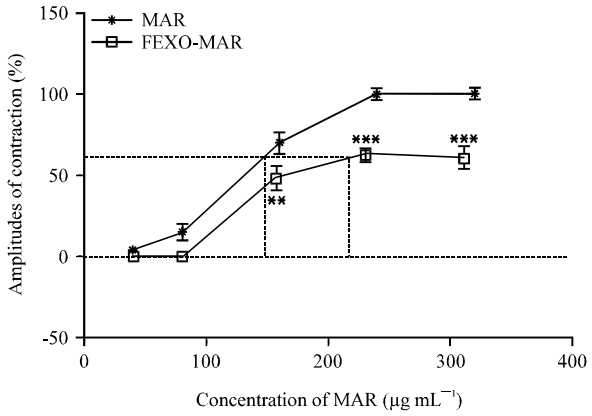


Fig. 4: Concentration-response curves for *M. micrantha* in isolated duodenum preparations of rabbit in the absence and presence of FEXO, Values are Mean±SEM (n = 4), **p<0.01 and ***p<0.001 compared to control

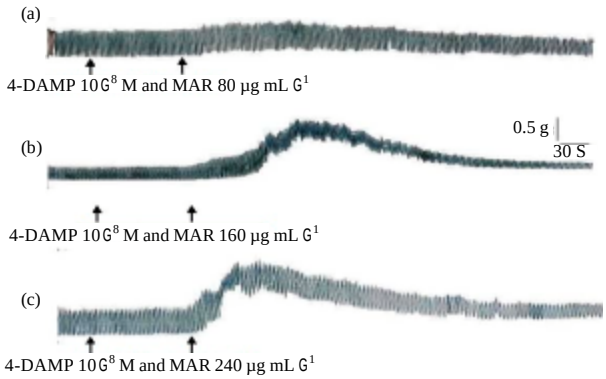


Fig. 5(a-c): Interaction effect between 4-DAMP and MAR on rabbit duodenum

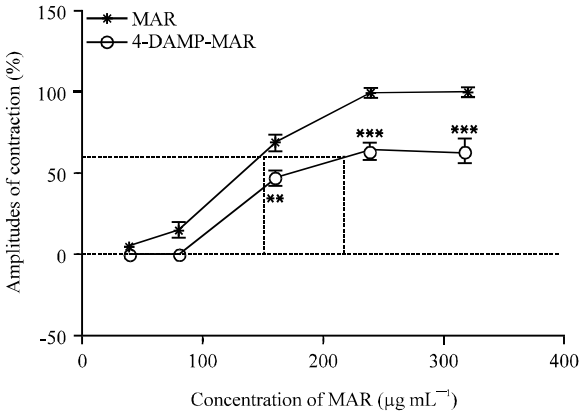


Fig. 6: Concentration-response curves for *M. micrantha* in isolated duodenum preparations of rabbit in the absence and presence of 4-DAMP, Values are Mean±SEM (n = 4), **p<0.01 and ***p<0.001 compared to control

**Effect of 4-DAMP on contraction elicited by the aqueous extract of *M. micrantha*:** The contractions in response to MAR (80-240 µg $mL^{-1}$) were decreased in the presence of the M3 antagonist 4-DAMP ($10^{-8}$ M) (Fig. 5), with an EC50 value of 211.11±4.01 µg $mL^{-1}$ as shown in Fig. 6. At 80 µg $mL^{-1}$, MAR-induced contraction was not significantly relaxed by application of 4-DAMP ($p>0.05$). But these decreases were significant ($p<0.01$-$0.001$) with the high concentrations of the extract (160 to 240 µg $mL^{-1}$).

## DISCUSSION

Based on the previous pharmacologic investigations, the aqueous extracts of *Mareya micrantha* has been reported to have spasmogenic activity in isolated tissues like ileum (Tsai *et al.*, 1995). To see whether the contractile effect of the aqueous extract is also mediated through histamine-like mechanism, rabbit duodenum was pre-treated with fexofenadine before the re-determination of the effect of test drug (MAR). It is well known that fexofenadine (anti-histamine) competes with histamine for H1 receptor located on cell wall (Weller *et al.*, 2012). This treatment has significantly blocked the contractile effect of the aqueous extract, similarly to that of histamine. This result indicates that the spasmogenic action of *M. micrantha* is mediated through a mechanism similar to that of histamine. Histamine is an important cellular messenger of the gastrointestinal tract (Schworer *et al.*, 1994) which stimulates various muscles including the gut tissues through activation of H1 receptor (Hill, 1990). Histamine acts through hydrolysis of phosphytidylinositol with a resultant increase in free cytosolic $Ca^{2+}$ (Dharmsthaphorn *et al.*, 1989; Wasserman *et al.*, 1988). The calcium released increases the peristaltic movements of the gut. The aqueous extract of *M. micrantha* probably contains histamine-like spasmogenic components acting through this mechanism.

We have also examined whether the contractile response of the plant extract is mediated via M3 muscarinic receptor. The plant extract was challenged in the presence of M3 antagonist 4-DAMP (An *et al.*, 2002). The stimulant effect of the aqueous extract was strongly blocked by M3 antagonist 4-DAMP, suggesting that MAR causes contractions of rabbit duodenum by the way of M3 receptor. It is well established that acetylcholine a neurotransmitter released by the parasympathic nervous system, mediates its actions in the gut by stimulation of M3 muscarinic

receptor subtype (An *et al*., 2002). Activation of M3 receptor releases calcium from intracellular stores such as sarcoplasmic reticulum, in an inositol triphosphate (IP3) dependent way (Ehlert *et al*., 1999; Okamoto *et al*., 2002).

Through this mechanism, ACh plays an important physiological role to regulate the peristaltic movement of the gut. The observed effect of aqueous extract similar to that of acetylcholine may explain the traditional use of MAR leaves in constipation. Experiments with the soy (Roeytenberg *et al*., 2007) and *Croton tiglium* L. (Hu *et al*., 2010, 2012) showed similar results. In fact, these plants had a contractile action on the guinea-pig and the rabbit isolated jejunum via activation of muscarinic receptors (M3 and M2).

In the present study, phytochemical investigations performed on the plant (MAR) revealed the presence of bioactive compounds such as phenolic compounds, alkaloids, flavonoids, saponosids, terpenoids, sterols and tannins. The presence of alkaloids (Neuwinger, 1996; Ghayur and Gilani, 2005) and polyphenols (Cimanga *et al*., 2010) as the plant constituents, which are known to possess gut stimulatory properties and laxatives activities, may explain the gut stimulatory action of the aqueous extract of *M. micrantha*.

Toxicological studies for all herbal medicine including the determination of their $LD_{50}$ are necessary (Abere *et al*., 2010). The $LD_{50}$ of the aqueous extract (MAR) is 566.66 mg $kg^{-1}$ b. wt. (calculation method) and 562.34 mg $kg^{-1}$ b.wt. (graphic method). According to toxic substances classification, *M. micrantha* is weakly toxic. Therefore, it can be qualified as relatively safe.

## CONCLUSION

In conclusion, contractions evoked by the plant extract would depend on both M3 and H1 receptors. Theses result may provide a rational basis to support its use in local folk medicine as laxative for the treatment of constipation. Further studies will be performed in order to isolate bioactive spasmogenic ingredients and indicate their mode of action.

## ACKNOWLEDGMENT

The authors are indebted to Professor Aké-Assi Laurent (Laboratory of Botany, Training and Research Unit-Biosciences, University of Cocody-Abidjan, Côte d'Ivoire) for botanical identification of Mareya micrantha (Benth.) Müll. Arg. (Euphorbiaceae).

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