



Inhibitory Effect of *Varthemia iphionoides* Extract on the Contractility of Isolated Rabbit Ileum

Yousef Salameh, Monther Abdel Fattah, Awni Abu-Hijleh, Ghaleb Adwan, Naser Jarrar and Kamel Adwan.

Department of Biology and Biotechnology, An-Najah National University, Nablus, Palestine.

Received on:20-08-2011; Revised on: 15-09-2011; Accepted on:10-11-2011

ABSTRACT

Varthemia iphionoides is indigenous to Palestine, where it is traditionally being used for treatment of gastrointestinal disorders. The aim of this paper was to provide pharmacological validation to this medicinal use. Water and ethanol extracts of *Varthemia iphionoides* were studied on isolated rabbit ileum for spasmolytic action. These extracts showed a reduction of the amplitude and the tone of spontaneous contraction in a concentration-dependent manner. Apart from that, water extract of *Varthemia iphionoides* drastically diminished the increase of the contraction of the ileum caused by acetylcholine (ACh) concentration of $5.5 \times 10^{-6} M$. This indicates that *Varthemia iphionoides* extract probably acts at least through muscarinic receptors blockade.

Key Words: *Varthemia iphionoides*, Medicinal plants, plant extracts, small intestine, antispasmodic effect

INTRODUCTION

The West Bank of the Palestinian territories encompasses a unique and rich habitat for a wide variety of plant life, most of which can be collected and studied efficiently in a relatively small land area. *Varthemia iphionoides* Boiss and Bl. is widely distributed in Palestine and neighboring countries. It is a perennial, bushy plant, 20–50 cm long, with a woody base and an aromatic, unbranching, hairy and sticky stems [1]. The aerial part of this plant is commonly used in the local folk-medicine for treatment of gastrointestinal disorders [2], the treatment of patients with diabetes mellitus [3] and healing eye inflammations [4]. Due to this extremely widespread application, the aim of this work is to determine the effects of the *Varthemia iphionoides* extract on the contractility of the longitudinal smooth muscle layer in the wall of rabbit ileum.

MATERIALS AND METHODS

Plant extract preparation

Leaves of *Varthemia iphionoides* were collected from the northern area of Palestine in March 2011. Identification and classification of the plant material was performed at the Faculty of Science, An Najah N. University. The dried leaves were made free of dirt and ground to powder. The ground leaves were soaked in hot water and 80% ethanol at room temperature for 48 h with occasional shaking. After filtering each solvent through a single layer of muslin cloth, the final filtrate was collected by passing it through a Whatman grade 1 filter paper. Filtrates were evaporated to dryness and weighed. Water and 80% ethanol, used for extraction, had no effect on tissue contractility in the control experiments.

Antispasmodic effect

The spasmolytic activity of the test compound was studied by using isolated rabbit ileum as described previously [5]. Respective segments of 20-30 mm long were suspended in 50 mL organ bath chamber filled with Tyrode's solution containing (in mM): 136 NaCl, 2.7 KCl, 1.4 CaCl₂, 2H₂O, 0.5 MgCl₂, 6H₂O, 11.9 NaHCO₃, 0.42 NaH₂PO₄ and 5.56 Glucose (bubbled continuously with 95% oxygen in carbon dioxide and maintained at 37°C) [6].

One end of the isolated segment was tied to a holder at the bottom of the bath, while the other was connected to lever of kymograph on which the contractions were recorded with a pen. Prior to the measurements, the ileal segments were allowed to equilibrate for 20 min under a resting tension of 1.0 g. Under these experimental conditions, rabbit ileum exhibits spontaneous rhythmic contractions, allowing testing of the relaxant (spasmolytic) activity directly without the use of any agonist.

To assess the mode of action of the antispasmodic effect of the test compound, acetylcholine (ACh) was used to depolarize the preparations. Addition of ACh to the tissue bath produced a sustained contraction. Relaxation of intestinal preparations by the compound, precontracted with ACh, was expressed as percent of the control response mediated by ACh.

Statistical analysis

Results were expressed as Mean \pm standard error of the mean (SEM). The data were statistically analyzed using student's t-test and $p < 0.05$ was considered to be significant.

RESULTS

The water and ethanol extracts prepared from *Varthemia iphionoides* were examined for spasmolytic activity on the rabbit ileum. Tracings the effects of water and ethanol extracts shown in Figure 1 indicates that various concentrations of ethanol extract (4.4 to 13.2 $\mu g/ml$) and water extract (60 to 168 $\mu g/ml$) produced a significant spasmolytic activity on the rabbit ileum, with maximum response being produced at 13.2 $\mu g/ml$ for ethanol extract and 168 $\mu g/ml$ for water extract. *Varthemia iphionoides* extract was completely reversed after washing the preparation, suggesting a non-toxic effect of *Varthemia iphionoides* at 13.2 $\mu g/ml$ and 168 $\mu g/ml$ concentrations for ethanol and water extracts, respectively.

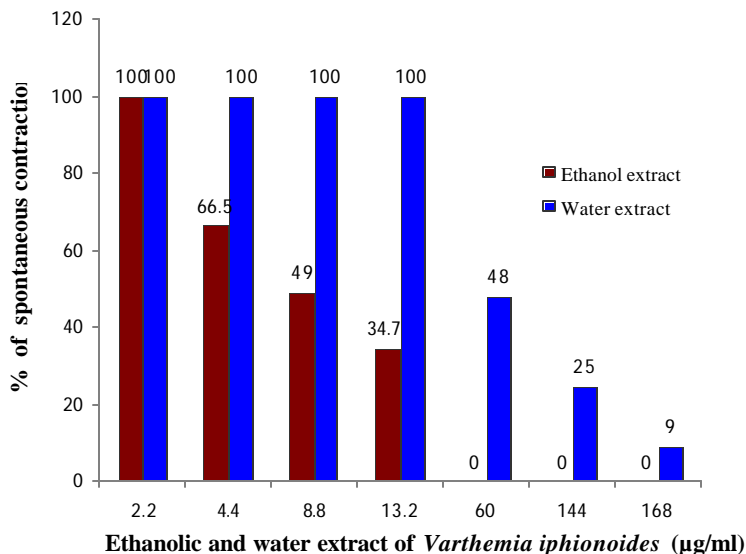


Figure 1. Effect of ethanolic and water extracts of *Varthemia iphionoides* on spontaneous contraction on isolated rabbit ileum.

*Corresponding author.

Kamel Adwan
Department of Biological Sciences,
An-Najah National University,
P.O. Box (7)-Nablus - Palestine
Tel phone: +970 9 234 5113-7
Fax: +970 9 234 5982
E-mail: adwank@najah.edu

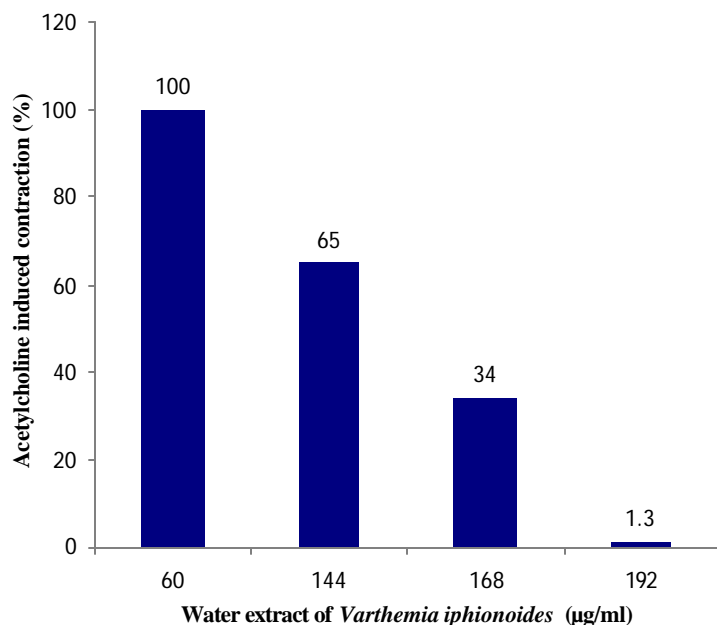


Figure 2. Effect of water extract of *Varthemia iphionoides* on Acetylcholine induced contraction of the isolated rabbit ileum (100% was taken as contraction induced by 5.5×10^{-6} M Acetylcholine). Values shown are mean \pm SEM, n=3.

To elucidate the mechanism of the effect of the extract used, ileal smooth muscle preparation was exposed for 10 min to Tyrode extract free and then was challenged with Ach. On the basis of our results, Ach caused an increase in the ileum basal tone. Maximal increase percentage (100%) was recorded in sections treated with ACh concentration of 5.5×10^{-6} M as expressed in Figure 2.

Water extract concentrations of 144, 168, and 192 µg/ml gave significant spasmolytic effects since they decreased the contraction respectively by $35 \pm 0.88\%$ ($p < 0.05$), $66 \pm 1.53\%$ ($p < 0.05$), and $98.7 \pm 0.33\%$ ($P < 0.05$).

DISCUSSION

Natural extracts have been proved to be useful in different human pathological conditions. The scientific consideration of the therapeutic potential of plant extracts is still inappropriate due to the lack of both pharmacological and epidemiological basic studies. Here, we examined the spasmolytic activity of water and ethanol extracts of *Varthemia iphionoides* on the rabbit ileum. The ethanol extract showed intense relaxation effects, approximately 65%, at 13.2 g/ml. The relaxation effects of water extracts were lower than those of ethanol extract. The possibility for this could be that all of the identified components from plants with relaxation effects on smooth muscles, aromatic or saturated organic compounds, are most often obtained through initial ethanol extraction [7].

In this study, pretreatment of ileum with water extract completely diminished the responses of Ach on the rabbit ileum in a dose-dependent manner. The leaves of *Varthemia iphionoides* have been known to contain flavonoids [1,2] and plants containing flavonoids possess inhibitory effects on smooth muscle activity [8,9].

Acetylcholine exerts its stimulatory effects in the gut through muscarinic receptors localized in smooth muscle cell membranes [10]. Tropic alkaloids belonging to the group of cholinergic medicants, act as ACh antagonists on muscarinic receptors [11]. Thus, it is possible to speculate that extract of the *Varthemia iphionoides*, might act as competitive inhibitors of a certain type of muscarinic receptors, flavonoids being the most likely candidates because of their abundance and structural similarities to tropic alkaloids.

The results presented in this report highlight the potential of *Varthemia iphionoides* extract as a source of relaxant and spasmolytic compounds. These results also confirm the traditional medicinal use in Palestine of *Varthemia iphionoides* for treatment of gastrointestinal disorders. Further work is presently under way to identify the structure of the extracted compounds and characterize their spasmolytic action mechanisms on the rabbit ileum.

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Source of support: Nil, Conflict of interest: None Declared