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Investigation of the Direct Effects of the Alcoholic Extract of *Elaeagnus angustifolia* L. (Elaeagnaceae) on Dispersed Intestinal Smooth Muscle Cells of Guinea Pig

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Abstract

The effect of the ethanolic extract of *Elaeagnus angustifolia* was tested on dispersed smooth muscle cells (SMC) of the guinea pigs. A slight contractile response was observed when SMC were treated with low concentrations of the extract. Pre-treatment of the SMC with ethanolic extract of *E. angustifolia* caused concentration dependent inhibition of acetylcholine-induced contractions of the SMC.

Keywords:

Elaeagnus angustifolia, dispersed smooth muscle cells, guinea pigs, acetylcholine.

Introduction

Elaeagnus angustifolia L. is a Mediterranean representative of the family Elaeagnaceae. The family Elaeagnaceae comprises three genera and about 50 species in North America and Eurasia as far southwards as Malaysia and Australia. In Jordan, *E. angustifolia* is the commonly found species. It is known with

the English common name "oleaster" and Arabic common name "zeizafoun". It is a shrub or tree, reaching up to 8m in height with branches often spiny. The leaves are 2-5 x 0.6-1.6cm, short petioled, lanceolate or lanceolate linear, light green above and silvery beneath. The flowers 4-8mm, short pedicelled, 1-3 in accillary clusters. The fruits are 2x1.5cm, ovoid and are yellowish with silvery scales. It blossoms from April to June [1]. Medicinal uses have been reported for different species of the genus *Elaeagnus* and for some isolated or identified substances from this genus. The ripe fruits of *E. philippinsis* have been used in the treatment of amoebic dysentery and the epigallactechin which was identified in *E. glabra* has been reported to exert antibacterial activity [2, 3]. The leaves and fruits of *E. angustifolia* are believed to have antipyretic effects [4, 5]. In Iranian traditional medicine, fruits of *E. angustifolia* have been used to relief pain in patients with rheumatoid arthritis [5]. In addition, flowers of oleaster are used to treat nausea, vomiting, flatulence, asthma and jaundice [6]. Also Lev and Amar (2002) have reported the use of the fruits of *E. angustifolia* in the treatment of dysentery and diarrhea in Jordan [7].

Few studies have been conducted to identify the biological activities of *E. angustifolia.* In humans, the pollen of *E. angustifolia* has shown allergenicity and minimal to moderate cross allergic reactivity with olive pollen [8]. In studies using animal models with induced inflammation, extracts from *E. angustifolia* fruits have been shown to display anti-inflammatory properties [9]. In another study Ahmadiani et al. (2000) demonstrated anti-nociceptive and anti-inflammatory effects for *E. angustifolia* fruit extracts [10]. The anti-nociceptive effects were established using chronic and acute pain models in rats and the anti-inflammatory effect using the paw inflammation model in rats. The demonstration of anti-nociceptive effects in hot-plate and writhing tests in mice following intraperitoneal injections of extract fractions and the relaxant activity in mice muscle may indicate inhibitory effects of *E. angustifolia* extracts on the physiological functions of excitable tissues [11, 12]. Gurbuz et al. (2003) found potent gastroprotective effect for the methanolic fruit extract of *E. angustifolia* in ethanol induced ulcer in rats based on histopathological

examination and determination of the ulcer index [13]. These reports initiated us to study the potential pharmacological effects of the leaf and flower extracts of *E. angustifolia* on the activity of gastrointestinal smooth muscle.

Experimental

Plant material

Aerial parts during the flowering stage of *E. angustifolia* were collected from the tree in the Campus of the University of Jordan. Plant material was identified by Prof. Dr. Barakat Abu Irmaileh, Faculty of Agriculture, University of Jordan. A voucher specimen has been kept at the Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Jordan. Afterwards the plant material was dried at room temperature and then submitted to the extraction process.

Extract preparation

After drying 100 g finely crushed and coarsely powdered leaves and flowers were extracted in a Soxhlett apparatus for six hour using 1lt 96% EtOH. The extract was kept overnight, filtered and used for the preliminary phytochemical test using thin layer chromatographic (TLC). For the *in vitro* experiments 1g of the completely dried ethanolic extract was dissolved in 10ml distilled water and used for the preparation of the final concentrations.

Screening of the extract by TLC

For the TLC screening, solvent systems and spraying reagents were prepared according to Wagner and Bladt [14]. Ready coated TLC plates (Silicagel 60 with fluorescence UV_{254} thickness 0.2mm; Albet, Spain) and analytical grade solvents were used. *E. angustifolia* extract was tested for the presence of several groups of secondary materials and gave positive color reactions for the flavonoids and terpenoids.

Dispersion of smooth muscle cells (SMC)

Fully anaesthetized guinea pigs weighing between 350-450g were sacrificed by opening of the chest cavity. The whole small intestine was removed and placed in freshly prepared Krebs solution (pH 7.4) and previously aerated with 95% O_2 and 5% CO_2. Small intestine was cut into small pieces of 2-2.5cm. After removal of longitudinal layer and mucosa by mechanical scraping, the circular layer has been taken and prepared for enzymatic digestion by chopping.

SMC were dispersed by enzymatic digestion as described by Murthy (1991) [15]. Briefly, chopped preparation was placed into 30ml Krebs solution containing 320U/ml collagenase. The digestion process continued for about 90minutes at 35^{o} C with very low bubbling by 95% O_2 and 5% CO_2. Aliquot, containing dispersed smooth muscle cells after the process of digestion, was filtered through nitex mesh of 500micrometers. The filtrate was centrifuged for 10 minutes at 3000 rpm. After this process, digestive solution was removed, and cells were resuspended in collagenase free Krebs solution for washing and centrifuged twice. After the second wash, cells were suspended into Krebs solution and ready for use in our study.

Experimental protocol

Equal volumes of solution containing dispersed cells were placed in test tubes. Then tubes were divided into control and extract treated groups. The control group included one blank tube containing only dispersed cells (C), and a second one Ach added dispersed cells (AchC) to give final concentration of $5x10^{-5}$M. In this group, muscle activity was stopped after 15 sec of Ach treatment by acrolein (1%). The extract treated groups comprised those treated only with extract (E) and Ach added group (EAch) after pretreatment with the extract for 45seconds. The final concentrations of the extract in the solutions with the dispersed cells were 1mg/ml, 100µg/ml and 10µg/ml. In both groups, after 15seconds of Ach addition to EAch, the muscle activity was stopped by addition of acrolein giving final concentration of

1%. For micrometric scanning, dispersed cells were spread over microscopic slides, covered with cover slide and kept overnight in humid atmosphere at 4^{o} C prior to the measurement.

Measurement of dispersed SMC

24 hours later, the length of SMC was measured by micrometric scanning technique as described by Murthy et al. [15]. The mean length of the first 50 encountered cells was taken from each slide and considered as one experimental result.

Analysis of results

All data are presented as means of smooth muscle length ± SEM. The differences between means of the control - blank (C) and acetylcholine treated cells (AchC) - were compared to the extract treated (E) and extract pre-treated samples (EAch) by using Student's *t*-test. Significance was considered when *p* value is <0.05.

Results and discussion

Figure 1 shows the mean length of SMC from guinea pig small intestine before Ach treatment. The mean length was 105.7 ± 1.3μm (n = 9). Induction of contraction by Ach ($5x10^{-5}$ M) caused a decrease in the length of smooth muscle cells to 69.7 ± 1.3μm (Fig 2). This indicates 34% decrease in the muscle length by treating the cells with Ach.

SMC response to Ach was significantly reduced when cells have been pre-treated with 1mg/ml ethanolic extract of *E. angustifolia*. The mean length of SMC in this group was 93.3 ± 6.1μm (n= 6). However, the cells pre-treated with lower concentrations of extract have not shown significant inhibition of their response to

Ach. In these groups, the mean length of cells was 76.0 ± 1.9µm (n = 6) and 69.7 ± 1.6µm (n = 6) when cells were pre-treated by extract concentrations of 100µg/ml and 10µg/ml respectively (Fig. 2). These observations indicate a dose dependent inhibition of the contractile response to Ach when SMC were pre-treated with ethanolic extract of *E. angustifolia.*

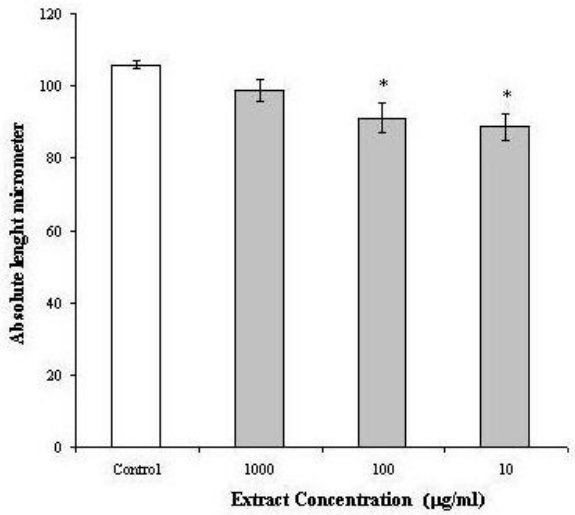

Fig. 1: The effect of *Elaeagnus angustifolia* L. extract on small intestinal smooth muscle cells.

The mean length of the muscle cells was also measured when cells have been treated with the extract alone. Treatment of cells with 1mg/ml of the extract has caused no significant changes in the mean length of muscle cells. In this group, the mean length of cells was 98.7 ± 3.0µm (n = 6). Conversely, a significant decrease in the mean length of muscle cells to 91.2 ± 3.9µm (n = 6), and to 88.7 ± 3.7µm (n = 7) was observed at extract concentrations of 100µg/ml and 10µg/ml respectively. This indicates a slight contractile response when SMC were treated with low concentrations of extract.

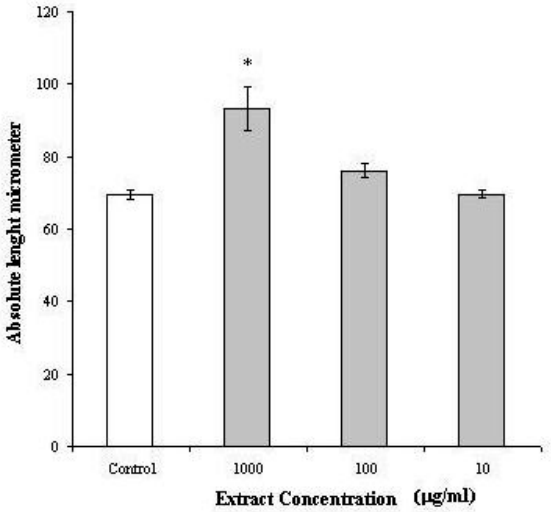

Fig. 2: The effect of acetylcholine on *Elaeagnus angustifolia* L. extract pre-treated intestinal smooth muscle cells.

Studying the effect of test substances on dispersed SMC has an advantage as we can eliminate SMC responses mediated via enteric nervous system. This may allow us to observe and evaluate direct effects of test substances on SMC.

In the present study, pre-treatment of SMC with ethanolic extract of *E. angustifolia* has significantly inhibited the response of SMC to acetylcholine. The contraction induced by Ach in pre-treated cells with 1mg/ml extract was only 33% of that induced in untreated cells. The inhibition of contractile response was decreased when cells were pre-treated with lower concentrations of extracts. In these groups, the contraction of cells was 83% and 100% of that induced by Ach in untreated cells at extract concentrations of 100µg/ml and 10µg/ml respectively (Fig. 2). The increased inhibition of SMC responses to Ach by increasing concentration

of extract strongly indicates that ethanolic extract from *E. angustifolia* has caused concentration dependent inhibition of Ach induced smooth cells contraction.

In this study, the effect of the extract alone on the SMC was also evaluated. We have observed a significant decrease in muscle length to 91.2 ± 3.9µm (p<0.05), and to 88.7 ± 3.7µm (p<0.05) by treating cells with extract concentrations of 100µg/ml and 10µg/ml respectively (Fig.1). This indicates presence of certain constituents in the ethanolic extract that can induce contraction of SMC. The absence of such effect in cells treated with high concentration of extract, may be due to the increased concentration of inhibitory constituents found in the extract which exert their activity at certain concentration.

The inhibition of isolated SMC activity by the extract may explain in part the beneficial use of *E. angustifolia* reported by Mirhyadar in the treatment of nausea, vomiting and flatulence [6]. These symptoms are motor related disturbances of gastrointestinal functions and the motor functions are under neural control. The reported anti-nociceptive activity of extract of *E. angustifolia* indicates also effects on the function of neural cells [10, 11]. It is difficult to demonstrate whether the beneficial effect of this extract to reduce motor related symptoms is due to direct effect on the contractile activity of the SMC or by mediated effects through neural control of gastrointestinal motor functions.

In addition, the study by Hosseinzadeh and his colleagues has also demonstrated relaxant effects on mice hind leg muscle [12]. In this study, the mechanism of the relaxant effect caused by extract was not identified. It was not clear whether the relaxation was induced by inhibitory effects of the extract on motor neurons that enervate muscle and inhibition of transmission at neuromuscular junction, or by affecting excitation contraction coupling at the skeletal muscle. All these studies indicatrd effects of the extract of *E. angustifolia* on excitable cells. Our study has clearly demonstrated that *E. angustifolia* extract

inhibited contractile responses by its direct effect on SMC as the effect through enteric neurons was eliminated by dispersion of cells.

Further investigations should be carried out to study the pharmacological effects of *E. angustifolia* extract on excitable cells including neurons, respiratory and vascular SMC and to clarify the mechanisms that are involved in the inhibition of SMC activity to extract treatment. Phytochemical investigations are needed to elucidate the nature of the substances involved in different biological activities of *E. angustifolia.*

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