

Preclinical Vascular Activity of an Aqueous Extract from Flowers of *Calendula officinalis*

Loipa Galán Martínez

Laboratorio de Electrofisiología Básica. Departamento Docencia-Investigaciones. Instituto de Cardiología y Cirugía Cardiovascular, 17 N°702, Vedado, La Habana, Cuba

Abstract: Various parts of *Calendula officinalis* have been used as a medicinal herb. This study was carried out to evaluate the effects of an aqueous extract from flowers of *Calendula officinalis* on vascular smooth muscle of rats. The effects on aortic contraction of the extract of *C. officinalis* at different concentrations were assessed in endothelium-denuded rat thoracic aortic rings pre-contracted with 60 mmol/L of KCl. The results demonstrated that the extract induced a concentration-dependent relaxation in endothelium-denuded rat aortic rings with an IC_{50} of 0.5 ± 0.01 mg/mL. In conclusion, this investigation has shown that the mode of action on the vasorelaxant effect caused by this extract is endothelium-independent. The findings from this study provide a scientific basis for the use of this plant in traditional medicine and merits further investigations.

Key words: *Calendula officinalis* Lin., herbal medicine, vasorelaxation.

1. Introduction

Calendula officinalis Lin. is an annual herb that is widely cultivated and can be grown all over the world and is commonly cultivated as an ornamental plant, popularly known as calendula herb, calypso orange florensis, claveton (Spanish), pot marigold, ruddles, common marigold or Scotch marigold (English) of the family Asteraceae or Compositae (family), cowbloom, death-flower, drunkard gold, Fiesta gitana Gelb, fior d'ogni (Italian), flaminquillo (Spanish), fleurs de tous les mois (French), among others names. Flowers were used as a medicinal herb, as well as a dye for fabrics, foods, and cosmetics [1].

A number of phytochemical studies have demonstrated the presence of several classes of chemical compounds, the main ones being terpenoids, flavonoids, coumarines, quinones, volatile oil, carotenoids and amino acids [1, 2]. The ether, butanol and water extracts, containing flavonoids, showed antioxidant activity [2, 3].

Main flavonoids in calendula are isorhamnetin and quercetin, isorhamnetin-3-O- β -D-glycoside, isoquercetin,

calendoflavoside, narcissi, isoquercitrin, rutin, quercetin-3-Orutinoside, quercetin-3-O-glucoside, isorhamnetin-3-O-rutinoside, isorhamnetin-3-O-2G-rhamnosyl rutinoside, neohesperidoside, isorhamnetin-3-O-neohesperidoside. The flavonoid content depends on the plant variety, time and place of cultivation, and there appears a relationship between floret color and total flavonoid content of *C. officinalis* [4].

Various parts of *Calendula officinalis* have been used in the popular medicine to treat a variety of ailments including abdominal cramps, constipation, fevers, cancer and as an emmenagogue [1, 5]. Traditionally, the decoction of flowers of *C. officinalis* has been used as wound-healing aid and topical anti-infective agent, as mouthwashes in stomatitis, and in pyorrhea; in the treatment of gastritis, from ulcers, hepatitis and other diseases gastrointestinal; in the treatment of hypertension, tachycardia and arrhythmia; in the treatment of various urinary system conditions as well as in diseases of the central and peripheral nervous system [6].

Studies had shown various pharmacological activities such as nephroprotective, hepatoprotective,

Corresponding author: Loipa Galán Martínez, PhD, research field: preclinical pharmacology.

hypoglycemic, hypolipidemic, and antioxidant potential of *C. officinalis* in experimental and clinical models [1, 2]. Other biological activities have been investigated such as immuno-stimulant activity, spasmogenic and spasmolytic activity, genotoxic and antigen-toxic activity, anti-bacterial and antifungal activity, among others [4].

In early animal studies, high doses of calendula preparations were reported to possess hypotensive effects [7].

An extract of calendula achieved cardioprotection in hearts of rats by stimulating left ventricular developed pressure and aortic flow as well as by reducing myocardial infarct size and cardiomyocyte apoptosis. Cardioprotection appears to be achieved by changing ischemia reperfusion-mediated death signal into a survival signal by modulating antioxidant and anti-inflammatory pathways [8].

The present study was carried out to evaluate the effects of an aqueous extract from flowers of *Calendula officinalis* on vascular smooth muscle of rats.

2. Materials and Methods

2.1 Plant Extract

Calendula aqueous extract was prepared according to previously described procedure [9, 10] by the Drug Research and Development Center (Centro de Investigación y Desarrollo de Medicamentos, CIDEM), Havana City, Cuba (Lot number 17004) and the quality control certification was provided. This is a raw material for the production of over-the-counter phytotherapy commercialized in Cuba.

2.2 Animals

Male adult (7-8 weeks) Wistar rats were obtained from the National Center for Laboratory Animal Reproduction (CENPALAB; La Habana). Prior to experiment, animals were adapted for seven days to laboratory conditions (controlled temperature 25 ± 2 °C, relative humidity $60\% \pm 10\%$ and 12 h

light/dark cycles). Tap water and standard diet for rodents supplied by CENPALAB were freely provided. All procedures were also conducted according to the European Commission guide-lines for the use and care of laboratory animals and approved by the Ethic Committee from the Institute of Cardiology and Cardiovascular Surgery. The minimum number of animals and duration of observation required to obtain consistent data were employed.

2.3 Aortic Rings

Thoracic aortic rings were dissected from rats under pentobarbital anesthesia. Care was taken to mechanically remove the endothelium with the purpose of verifying the direct actions of the tincture on vascular smooth muscle. They were fixed to a force transducer and placed in bath chamber continuously perfused (10 mL/min) with Tyrode solution of the following composition (mmol/L): 140 NaCl, 2.5 KCl, $MgCl_2$, 2 $CaCl_2$, 10 Trishydroxymethylaminomethane, 10 glucose (pH = 7.4, gassed with O_2 ; $T = 35$ °C) and stabilized, under a load of 0.8 g, for 30 min before the beginning of the experiment, according to the procedure of Galán et al. [11] with slight modifications. Contraction was induced by replacing NaCl by KCl (60 mmol/L). After an equilibration period of 30 min, the endothelium removal was confirmed by the administration of acetylcholine (10 μ mol/L) to precontracted vascular rings. A set of experiments were conducted using cumulative addition of extract (0.03, 0.1, 0.3, 0.5, 0.7, 1 and 1.5 mg/mL) at 10-minute interval between successive additions. In the control experiments the vehicle had no effect on vascular contractility.

2.4 Statistical Analysis

Results are expressed as means and standard errors of means. Student's *t*-test evaluated the statistical significance for paired samples, previously checked that the data complied with the premise of normality.

Differences were considered statistically significant for $p < 0.05$. The graphics and the statistical

processing were done using the software OriginPro 8 SRO v8.0724 (MA, USA).

3. Results and Discussion

The extract of flowers of *Calendula officinalis* induced a concentration-dependent relaxation in endothelium-denuded rat aortic rings pre-contracted with 60 mmol/L of KCl (Fig. 1).

Concentration-response curve, based on the Hill function, was fitted to the experimental data obtained after applying aqueous extract of *C. officinalis* concentrations from 0.03 to 1.5 mg/mL with an IC_{50} of 0.5 ± 0.01 mg/mL (Fig. 2). The highest concentration studied (1.5 mg/mL) caused a 100% of vasodilatation, while the lowest concentrations (0.03 to 0.3 mg/mL) hardly caused vasodilatation. At 1 mg/mL a vasodilatation of $92.87\% \pm 1.2\%$ occurred (Fig. 2).

These findings suggest that the relaxation effect of this aqueous extract of *C. officinalis* on rat aortic rings pre-contracted with 60 mmol/L of KCl is endothelium-independent.

Although further studies are needed to see if the chemical components of this extract have any direct effect on calcium channels, the decrease of force of vascular contraction by this extract should be at least partly due to an inhibition of voltage-gated calcium channels. This is because the contraction of vascular smooth muscle caused by high K^+ solution (60 mmol/L) containing calcium is associated with an increased entry of extracellular calcium through voltage-gated calcium channels [12].

According with the present results, Bashir et al. [5] showed that the aqueous and ethanolic floral extracts of *C. officinalis* flowers, when assayed in rabbit jejunum, caused a dose-dependent relaxation of spontaneous and K^+ induced contraction; further fractionation of the extract with dichloromethane showed inhibition of spontaneous contractions in a dose range of 0.01-0.03 mg/mL and this is 10 times more potent than the parent crude extract. This study indicated that spasmolytic activity was primarily due to blocking

effects on calcium channel.

The hypotensive effects of calendula in animal studies [7] may be due in part to the vasorelaxant action shown in the present results.

Main flavonoids in calendula are isorhamnetin [4] and quercetin [4, 10]. The vasorelaxant effect of this aqueous extract of *C. officinalis* could be due to the quercetin present in the extract. Previous reports showed that quercetin promotes endothelium-independent vascular relaxation [13, 14] and Cogolludo et al. [15] indicated that quercetin produced relaxant effects in rat coronary arteries. Hou et al. [16] demonstrated that calcium channel block by quercetin along with potassium channel stimulation accounts for its spasmolytic effects in rat coronary artery preparations. Isorhamnetin is the metabolite of quercetin and also showed vasodilator effects that are not modulated by endothelial factors [13].

Other phytochemicals present in this aqueous extract of *C. officinalis* can also influence the response. So, the mixture of components present may have synergistic vasorelaxant actions.

An extract of calendula achieved cardioprotection in hearts of rats by stimulating left ventricular developed pressure and aortic flow as well as by reducing myocardial infarct size and cardiomyocyte apoptosis. Cardioprotection appears to be achieved by changing ischemia reperfusion-mediated death signal into a survival signal by modulating antioxidant and anti-inflammatory pathways [8]. Under diabetes condition also a cardioprotective effect of marigold hydroalcoholic extract was shown [17].

Besides the acute and subchronic toxicities of aqueous extract from flowers of *C. officinalis* by oral route in Wistar rats are low [10]. Silva et al. [18] also showed non-toxic effects of a hydroalcohol extract of *C. officinalis* flowers in rodents by the oral route, although there was evidence of renal and liver overload in a subacute study.

The results obtained with the aqueous extract of *C. officinalis* suggest that the plant may have some hypotensive potential.

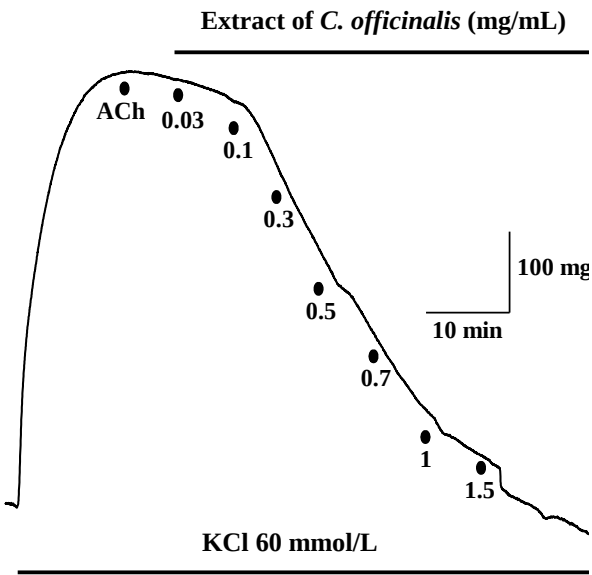


Fig. 1 Examples of the vasorelaxant effect of extract of flowers of *Calendula officinalis* at different concentrations in a rat aortic ring pre-contracted with 60 mmol/L of KCl. The endothelium removal was confirmed by the administration of acetylcholine (ACh, 10 µmol/L) to precontracted vascular rings.

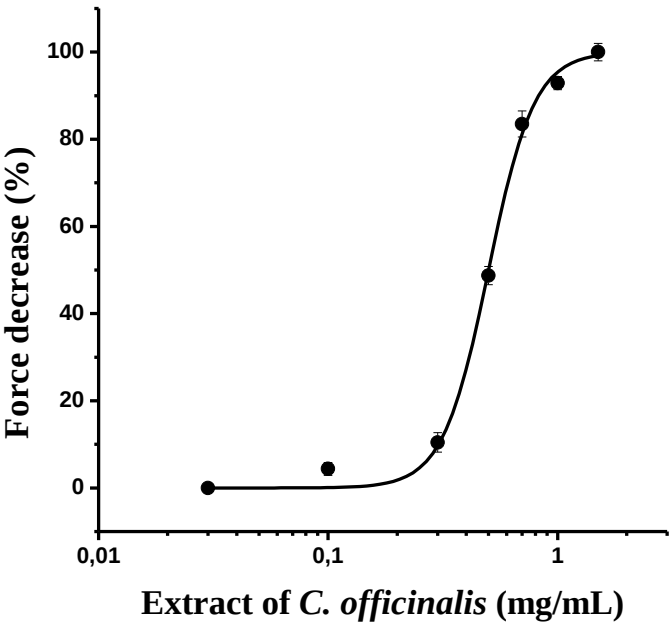


Fig. 2 Concentration-response curves for the inhibition of force of contraction by the extract of flowers of *Calendula officinalis* on KCl (60 mmol/L) induced contraction in endothelium-denuded rat thoracic aortic rings. Values are shown as means $\pm$ standard errors of means with $n = 5$ rats. Experimental data were fitted to a Hill function.

4. Conclusions

This study has shown that the mode of action on the vasorelaxant effect caused by this aqueous extract of flowers of *C. officinalis* is endothelium-independent. The results obtained with this extract suggest that it may have a potential clinical use for hypertension treatment; however, further *in vitro* and *in vivo* studies as well as the isolation of pure molecules should be carried out to find the exact mechanisms of actions for better scientific evidences. The findings are in accord with the ethnopharmacology use of this specie and the complementary clinical studies can support investigations assessing their use as antihypertensive agent.

Acknowledgments

This study was carried out in the laboratory of Basic Electrophysiology of Institute of Cardiology and Cardiovascular Surgery. This work was supported by the Cuban Ministry of Public Health (Research Project N°1903001).

References

- [1] Basch, E., Bent, M. S., Foppa, I., Haskmi, S., Kroll, D., Mele, M., et al. 2006. "Marigold (*Calendula officinalis* L.): An Evidence-Based Systematic Review by the Natural Standard Research Collaboration." *Journal of Herbal Pharmacotherapy* 6 (3/4): 135-159.
- [2] Verma, P. K., Raina, R., Agarwal, S., and Kour, H. 2018. "Phytochemical ingredients and pharmacological potential of *Calendula officinalis* Linn." *Pharmaceutical and Biomedical Research* 4 (2): 1-17.
- [3] Muley, B. P., Khadabadi, S. S., and Banarase, N. B. 2009. "Phytochemical Constituents and Pharmacological Activities of *Calendula officinalis* Linn (Asteraceae): A Review." *Tropical Journal of Pharmaceutical Research* 8 (5): 455-65.
- [4] Jan, N., Andrabi, K. I., and John, R. 2017. "*Calendula officinalis*—An Important Medicinal Plant with Potential Biological Properties." *Proceedings of the Indian National Science Academy* 83 (4): 769-87.
- [5] Bashir, S., Janbaz, K. H., Jabeen, Q., and Gilani, A. H. 2006. "Studies on Spasmogenic and Spasmolytic Activities of *Calendula officinalis* Flowers." *Phytotherapy Research* 20: 906-10.
- [6] Lastra, H., and Piquet, R. 1999. "*Calendula officinalis*." *Revista Cubana de Farmacia* 33 (3): 188-94.
- [7] Bojadjev, C. 1964. "On the Sedative and Hypotensive Effect of Preparations from the Plant *Calendula officinalis*." *Nauchni Trudove Nauchni Trud Visshia Meditsinski Institut, Sofia* 43: 15-20.
- [8] Ray, D., Mukherjee, S., Falchi, M., Bertelli, A., Braga, P. C., and Das, D. K. 2010. "Amelioration of Myocardial Ischemic Reperfusion Injury with *Calendula officinalis*." *Current Pharmaceutical Biotechnology* 11 (8): 849-54.
- [9] Carmona, R., Darío, O., González, M. L., and Muñoz, A. 2006. "Optimización de un proceso de obtención de extracto acuoso de *Calendula officinalis* Lin." *Revista Cubana de Plantas Medicinales* 11 (3-4). http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S1028-47962006000300006&lng=es&nrm=iso&tlng=es.
- [10] Lagarto, A., Bueno, V., Guerra, I., Valdés, O., Vega, Y., and Torres, L. 2011. "Acute and Subchronic Oral Toxicities of *Calendula officinalis* Extract in Wistar Rats." *Experimental and Toxicologic Pathology* 63: 387-91.
- [11] Galán, L., Talavera, K., Vassort, G., and Álvarez, J. L. 1998. "Characteristics of Ca^{2+} Channel Blockade by Oxodipine and Elgodipine in Rat Cardiomyocytes." *European Journal of Pharmacology* 357: 93-105.
- [12] Ghosh, D., Syed, A. U., Prada, M. P., Nystoriak, M. A., Santana, L. F., Nieves-Cintrón, M., et al. 2017. "Calcium Channels in Vascular Smooth Muscle." *Advances in Pharmacology* 78: 49-87.
- [13] Pérez-Vizcaino, F., Ibarra M., Cogolludo, A. L., Duarte, J., Zaragoza-Arnáez, F., Moreno, L., et al. 2002. "Endothelium-Independent Vasodilator Effects of the Flavonoid Quercetin and Its Methylated Metabolites in Rat Conductance and Resistance Arteries." *The Journal of Pharmacology and Experimental Therapeutics* 302 (1): 66-72.
- [14] Galán-Martínez, L., Herrera-Estrada, I., and Fleites-Vázquez, A. 2018. "Direct Actions of the Flavonoids Naringenin, Quercetin and Genistein on Rat Cardiac and Vascular Muscles." *Journal of Pharmacy & Pharmacognosy Research* 6 (3): 158-66.
- [15] Cogolludo, A., Frazziano, G., Briones, A. M., Cobello, L., Moreno, L., Lodi, F., et al. 2007. "The Dietary Flavonoid Quercetin Activates BKCa Currents in Coronary Arteries via Production of H_2O_2 . Role in Vasodilatation." *Cardiovascular Research* 73: 424-31.
- [16] Hou, X., Liu, Y., Niu, L., Cui, L., and Zhang, M. 2014. "Enhancement of Voltage-Gated K^+ Channels and Depression of Voltage-Gated Ca^{2+} Channels Are Involved in Quercetin-Induced Vasorelaxation in Rat Coronary Artery." *Planta Medica* 80: 465-72.
- [17] Ebrahimi, E., Shirali, S., and Talaei R. 2016. "The

Protective Effect of Marigold Hydroalcoholic Extract in STZ-Induced Diabetic Rats: Evaluation of Cardiac and Pancreatic Biomarkers in the Serum.” *Journal of Botany* Volume 2016, Article ID 9803928, 6 pages. <http://dx.doi.org/10.1155/2016/9803928>.

[18] Silva, E. J. R, Gonçalves, E. S., Aguiar, F., Evêncio, L. B., Lyra, M. M. A., Coelho, M. C. O. C., et al. 2007. “Toxicological Studies on Hydroalcohol Extract of *Calendula officinalis* L.” *Phytotherapy Research* 21: 332-6.