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SHORT COMMUNICATION

Inhibition of Intestinal Motility by Methanol Extracts of *Hibiscus sabdariffa* L. (Malvaceae) in Rats

A. M. Salah,[1]* J. Gathumbi[2] and W. Vierling[3]

[1]National Center for Natural Product Research, Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, University, MS 38677, USA
[2]Institute of Veterinary Medicine, University of Munich, Munich, Germany
[3]Institute of Pharmacology and Toxicology, Technical University of Munich, Munich, Germany

The methanol extracts of *Hibiscus sabdariffa* ($p < 0.01$) showed a significant dose dependent relaxant effect (IC_{50} = 350 μM) on rat ileal strip comparable to the effect shown by nifedipin and papaverine as reference compounds. Similarly, the extract when administered intraperitoneally significantly ($p < 0.05$–0.01) reduced the intestinal transit (13%–35%) in rats (IC_{50} = 250 μM). The extracts (40% ± 04%) and nifedipin (51% ± 05%) also potentiated the diarrhoea inducing effect of castor oil (IC_{50} = 350 μM). It is postulated that these effects are possibly generated by constituents such as quercetin and eugenol via a Ca^{2+} channel modulated mode of action. Copyright © 2002 John Wiley & Sons, Ltd.

Keywords: Hibiscus sabdariffa; Malvaceae; relaxant effect..

INTRODUCTION

Hibiscus sabdariffa L. (Malvaceae) is cultivated in the tropical and subtropical regions as an annual ornamental plant and also for export to Europe. In Cameroon and neighbouring regions of Africa, the fleshy calyx and leaves are cooked as a side-dish, potashes and for making jelly. In African folk medicine, the aqueous extracts are used as a digestive, antihypertensive, mild laxative and to control fever (Williamson, 1943; Wagner, 1999). The results of recent pharmacological studies clearly indicate that *Hibiscus sabdariffa* extract relaxed the isolated aortic ring (Owolabi *et al.*, 1995) and the jejunum in dogs (Ali *et al.*, 1991). The present study is aimed at investigating the effect and the mode of action of its extract in the intestinal motility in rats.

MATERIALS AND METHODS

The plant drugs and extraction. The plant (flowers of *Hibiscus sabdariffa*) was obtained from a local pharmacy in Munich, Germany. Dried flowers were milled to a fine powder by a high speed grinding mill. About 50 g of the fine powder was extracted with methanol at 60 °C soxhlet for 4 h. 1.5 g of the methanol extract was obtained and was dissolved in water and heated on a water bath at 60 °C for 30 min and filtered to remove the chlorophyll. The filtrate was rotavaporized yielding 85 g of the extract.

* Correspondence to: A. M. Salah, National Center for Natural Product Research, School of Pharmacy, University of Mississippi, University, MS 38677, USA.
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TLC and HPLC fingerprint analysis. The extract was co-chromatographed with flavonoid test samples (rutin, chlorogenic acid, hyperoside and isochlorogenic acid), luteolin and luteolin-7-glucoside, quercetin, isoquercetin, delphinidin and caffeic acid using ethyl acetate–formic acid–glacial acetic acid–water (100:11:11:26) as the mobile phase and precoated silica gel 60 F_{254} (20 × 20, thickness 0.25 mm Merck, Darmstadt, Germany) as the stationary phase. The plate was first observed at UV_{254} nm, sprayed with natural products–polyethylene glycol reagent (NP/PEG) and evaluated at UV_{366} nm.

HPLC was carried out with an HP 1090 A liquid chromatograh and an HP 1040 photodiode array with a Hewlett Packard detector on a LiChrospher 100 RP 18 (5μm) column 125 × 4 mm (Merck, Darmstadt, Germany), and a precolumn LiChrospher RP-18 4–4 mm (5 μm) (Merck, Darmstadt, Germany), with detection at wavelengths 210 nm, 254 nm, 280 nm and 366 nm. The mobile phase used for the separation was distilled water (solvent A) and acetonitrile (solvent B), all acidified with 33 μL of phosphoric acid (85%). It was started at 10% of B and a linear gradient of 10–30% MeCN during 20 min for a total run of 30 min, at a flow rate of 1 mL/min, and a volume of 12.5 μL of 1 mg/mL of the MeOH extract of *Hibiscus sabdariffa* was injected.

Animals. Swiss albino rats of either sex weighing 180–220 g bred in the animal house of the Technical University of Munich, Germany were used.

Ileal strip. The ileal strip was prepared according to Ambache (1954) and mounted vertically for isometric force recording and inserted into a two-chambered 50 mL bath containing a constantly aerated carbogen (5% CO_2 in O_2) and Krebs–Hanseleit solution at 35 °C. The muscles were stretched to obtain a resting force of 1.0 mN.

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The muscle was stimulated through punctuate electrodes at a frequency of 0.1 Hz by a square-wave pulse of 1–12 ms duration and at an intensity producing maximum contractions which had forces of 10–30 mN. The addition of the plant extracts was started after a 1 h equilibration period, after the addition of choline chloride (20 μmol/L) to prevent a relaxant effect owing to a progressive diminution of the choline content of the muscle (Reiter, 1981). Papaverine, and nifedipin, relaxant agonists along with the extract were used as a positive reference to antagonize the effects of $CaCl_2$-induced contractions.

Small intestinal transit. Charcoal food was given to two groups of six animals each after six h fasting but with free access to water. In group 1, the food was administered to animals intragastrically 70 min after the intraperitoneal injection of the plant extract. Controls were not treated with the plant extracts before receiving the charcoal food.

In group 2, diarrhoea was induced by oral administration of castor oil (0.5 mL) and the extract (350 μM), yohimbine (10 μM) and nifedipin (50 μM) 70 min were injected before castor oil administration. Control rats were not pretreated before the administration of castor oil.

The animals were killed after 30 min and the small intestine was rapidly removed and laid out on white filter paper for inspection and measurement of the distances traversed by the charcoal food. This distance was calculated as a percentage of the intestine length.

Evaluation of the response. Diarrhoea was expressed as a total score and the chi-square test was used to determine the significance between groups. Intestinal transit was expressed as the mean ± SE and Student's *t*-test was used to determine the significance of the different means. Values of $p < 0.05$ were considered statistically significant. Graphs were computer analysed using Prism 2.01 (Graph pad software).

RESULTS AND DISCUSSION

Chemical verification using TLC and HPLC fingerprint analysis revealed the presence of flavonoids (quercetin, luteolin and its glycoside) as well as chlorogenic acid in addition to other chemical compounds already known from this plant, namely: anthocyanins- delphinidin-3-glucosyl-xyloside (hibiscin) and cyanidin-3-glucosyl-xyloside (Sankara and Nair, 1972; Pouget *et al.*, 1990). Some flavonoids (gossypetin, hibiscetin and their respective glycosides) (Sankara and Nair, 1972), phenols and some phenolic acids (protocatechuic and pelargonidic acid (Tseng *et al.*, 1998), eugenol (Chen *et al.*, 1998) and some plant sterols (*β*-sitosterol) (Osman *et al.*, 1975) and ergosterol (Salami and Ibrahim, 1979) were also identified.

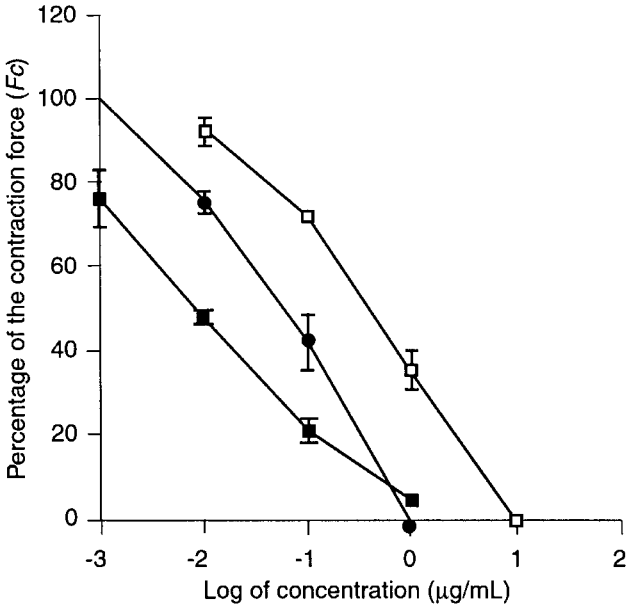

Figure 1. The relaxant effect of the MeOH extracts of *Hibiscus sabdariffa* of electrically precontracted ileal strip of guinea-pig in comparison with standard agonists (nifedipin and papaversine). ■, papaverine; ●, nifedipin; □, MeOH extract of *Hibiscus Sabdariffa*.

The extract significantly ($p<0.01$) inhibited the phasic contractions of the electrically stimulated ileal strips in a dose dependent manner (IC_{50} 350 μg/mL) similar to the reference drugs (papaverine and nifedipin) (Fig 1) but did not affect the $CaCl_2$-induced contractions. It also inhibited small intestinal transit in a dose dependent manner (Table 1). At higher doses the effect became statistically significant ($p<0.05$–0.01) with an inhibition of 13%–35% compared with the vehicle control. Treatment of the animals with yohimbine antagonized (54% ± 2%) whereas nifedipin (51% ± 05%) and the plant extracts (40% ± 4%) potentiated the diarrhoea inducing effect of castor oil ($p<0.05$–0.01) (Table 2). Studies showed that the extracts relaxed the papillary muscle (IC_{50} = 75.5 μM) at 0.1 Hz under the influence of 3 μM noradrenaline and 1 μM ryanodine (Salah *et al.*, 2000) suggesting a mode of action via inhibition of the Ca^{2+} influx through receptor operated channels. The fact that the extracts failed to affect $CaCl_2$ precontracted muscles indicates an inhibition of the release of Ca^{2+} from the intracellular stores.

The calcium channel blocking activity by the extract of *Hibiscus sabdariffa* could be due to some chemical substances present in this extract; quercetin at IC_{50} = 17.8 μM on the rat aorta (Morales and Lozoyta, 1994) and eugenol at IC_{50} = 224 μM on the guinea-pig papillary

Table 1. Effects of the MeOH extracts of *Hibiscus sabdariffa* on intestinal transit, expressed as distance travelled by the charcoal food as % of the total intestinal length. Values are mean ± SE of five experiments carried out with six animals each ($p < 0.05$–0.01) (Student's *t*-test)

Concentration (μM) of the plant extract	30	100	300	1000
Control (untreated animals) (A)	46 ± 5.1	45 ± 6.0	46 ± 5.0	47 ± 6.0
MeOH extracts of *Hibiscus sabdariffa* (B)	45 ± 5.0	32 ± 4.0	14 ± 1.3	12 ± 1.7
Inhibition effect (A−B)	1 ± 0.01	13 ± 2.0	32 ± 4.7	35 ± 4.3

Table 2. Effects of the plant extract in comparison with nifedipin and yohimbine on animals pretreated with castor oil on intestinal transit, expressed as distance travelled by the charcoal food as % of the total intestinal length. Values are mean ± SE of five experiments carried out with six animals each ($p < 0.05–0.01$) (Student's *t*-test)

	Treatment (B)		
Control (A)	Yohimbine 10 (μM)	Nifedipin 50 (μM)	MeOH Ext of *Hibiscus sabdariffa* (350 μM)
110 ± 17	56 ± 15	171 ± 13	160 ± 13
The potentiated or antagonized value in % (A–B)	54 ± 0.2	−51 ± 0.5	−40 ± 0.4

muscle (Neuhaus *et al.*, 1993; Sensch *et al.*, 1993) inhibit calcium influx from the extracellular space into the cell by binding at various sites on the L-type channels depending on their respective chemical structures. It could also have resulted from a synergistic effect of several other constituents which inhibited receptor-operated calcium entry into cells.

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