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Antispasmodic Effects of Hydroalcoholic Extract from *Gochnatia polymorpha* sp. *floccosa* in the Guinea Pig Ileum

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ABSTRACT

The effects of hydroalcoholic extract from the leaves of *Gochnatia polymorpha* sp. *floccosa* on the contractile responses of the isolated guinea-pig ileum were investigated using a force displacement transducer amplifier connected to a physiograph. Isolated ileum preparations were set up for recording of isometric contractions in 5 mL jacketed organ baths containing oxygenated Krebs-Henseleit solution at 37°C under 1 g of load. *G. polymorpha floccosa* extract inhibited the contractile responses in a dose-dependent manner against different agonists. The profile of inhibition was noncompetitive to acetylcholine, histamine, serotonin and bradykinin-induced contractions. IC_{50}s (and maximal inhibitions) values obtained were 247 μg mL^{-1} (83.1%), 818 μg mL^{-1} (72.62%), 450 μg mL^{-1} (75.82%) and 210.09 μg mL^{-1} (97.35%), respectively. These findings support the popular use in folk medicine of this plant as an antispasmodic on smooth muscles contractions.

Key words: *Gochnatia polymorpha*, antispasmodic, medicinal plant, guinea-pig ileum

INTRODUCTION

Gochnatia polymorpha, is a tree from *floccosa* subspecie, Asteraceae (Less) Cabr. family, popularly known as cambará-do-mato, cambará-guaçu or candeia that occurs in the south of Brazil. It's a tree of medium size, which can grow to ten meters in high (Lorenzi, 2002).

Among ethnopharmacological utilities of *G. polymorpha,* its leaves are used in Brazilian folk medicine to prepare teas and syrups against influenza cold, cough and other respiratory and gastrointestinal diseases (Correa, 1984; Mors *et al*., 2000). There are rare scientific citations about biological properties of this plant (Moreira *et al*., 2000; Stefanello *et al*., 2006a). Recent chemical and pharmacological researches revealed the presence of compounds with therapeutic potential (Bohlmann *et al*., 1986; Moreira *et al*., 2000; Stefanello *et al*., 2006b). The ethanolic extract of leaves from *G. polymorpha* it presented a significant antiinflammatory effect in oedema model. In the carrageenan-induced paw oedema test in rats, isolated pure compounds found in this plant, such as caffeic and chlorogenic acids, the aminoacid 4-hidroxy-N-metylproline and the flavonoids 3-O-metylquercetin, hiperoside and rutine were tested (Moreira *et al*., 2000). The antiinflammatory activity was described to all identified compounds (Jenkins *et al*., 1993;

Wu *et al.*, 1994; Chen *et al.*, 1995; Simoes *et al.*, 2004). Bohlmann *et al.* (1986) described the presence of two bisabolene derivatives and four dimeric guaianolides in the roots and in aerial parts was obtained two dimeric guaianolides in an *G. polymorpha* specie.

G. polymorpha barks possess potential therapeutic application in diseases caused by Gram-positives bacteria. In a Brazilian study, was verified an inhibitory activity of leaves extract of this plant against *Staphylococcus aureus* (Stefanello *et al.*, 2006b).

In order to obtain more detailed information about this plant, we explored an *in vitro* effect of the hydroalcoholic extract from the leaves, based in the possibility that this plant could be relaxant or antispasmodic on smooth muscle tissues.

MATERIALS AND METHODS

Plant: The botanical material of *G. polymorpha floccosa* was collected in Bom Retiro City, in the State of Santa Catarina, Brazil, September 2008. It was identified by Dr. Roseli Bortoluzzi (Universidade do Estado de Santa Catarina) and a voucher specimen was stored in the Herbarium Lages da Universidade do Estado de Santa Catarina, in Lages, Santa Catarina, under number 1858.

Preparation of *Gochnatia polymorpha* extract: From air-dried triturated leaves, the extract was gotten with 50% methanol (MeOH)-water in the proportion of 1:3, being macerated at room temperature (25±3°C) for 15 days. The solvent was evaporated in vacuum giving a residue, with extraction yields about 35 mg to each 100 g of material plant, which was dissolved in dimethyl sulfoxide (DMSO) 25% and later diluted in phosphate buffered solution to the desired concentration just before pharmacological use.

Animals: Albino guinea pigs of 2 to 3 months (300-400 g) were kept in automatically controlled temperature conditions (22±2°C), in 12 h light-dark cycles and with food and water *ad libitum*. All procedures were carried out according to the guidelines for animal experimentation of the American Association for Laboratory Animal Science (Trowning Manual Series) (Stark and Ostrow, 1991).

Isolated guinea pig ileum: Animals were killed after deep anesthesia of pentobarbital (100 mg kg^{-1} i.p.) by cervical dislocation and the ileum was carefully removed from the exposed abdominal cavity. Ileum strips of about 10 to 20 mm in length were taken from the portion situated 15 cm proximal to the ileum-caecal junction. The intestinal content was removed by washing with warmed nutritive Kreb's Henseleit solution and the mesenteric residues were eliminated. Preparations were set up for recording isotonic contractions under 1 g of load in 5 mL jacketed organ bath containing nutritive solution at 37°C, continuously bubbled with a mixture of 95% O_2 and 5% CO_2. The composition of the Kreb's Heinseleit solution was (mM): NaCl 118.0; KCl 4.4; $CaCl_2$ 2.5; $NaHCO_2$ 25.0; $MgSO_4$ 1.1; KH_2PO_4 1.2 and glucose 11.0 (Schlemper *et al.*, 2005). Preparations were allowed to equilibrate for at least 60 min before drug addition, during which the bath solution was replaced every 15 min. Isometric tension changes were recorded by means of Letica TRI 210 force transducers on a physiograph Powerlab ML 785. Cumulative concentration-response curves were obtained for contracturant agonists, which are excitatory neurotransmitters present in the gut (Campbell, 2009; Morini *et al.*, 1993; Chan and Rudd, 2006), such as acetylcholine (1 pM to100 μM), histamine (0.01 nM to100 μM), serotonin (0.1 pM to 100 μM) and bradykinin (0.01 to 3 μM) in the absence or in presence of the increasing *G. polymorpha floccosa* extract concentrations (100 to 1000 μg mL^{-1}), incubated to the preparations for 20 min beforehand.

In the maximal, three cumulative concentration-response curves were obtained for each preparation, with a 20 min rest between each curve. The maximal average response obtained from the first cumulative concentration–response curve (in the absence of extract) was taken as the 100% response value. In order to correct for spontaneous and/or vehicle-induced desensitization, control experiments were performed for all agonists tested, en separate sets of experiments, in the presence of corresponding concentration of vehicle. Control experiments demonstrated that the solvents used to dissolve plant extract did not affect the contractile response of the isolated tissues at the final bath concentrations.

Statistical analysis: The data are shown as Mean±SEM, except for the IC_{50} (concentration of drugs causing half-maximal responses), which are presented as geometric mean accompanied by their respective 95% confidence intervals. The statistical analyses were obtained by the ANOVA test, followed by the Dunnett's test when necessary. $P< 0.05$ or less was considered significant. The IC_{50}s were read starting from individual experiments for graphic interpolation on semi-logarithmic paper and analysis using the GraphPad InStat program.

RESULTS

The previous incubation of hydroalcoholic extract of *G. polymorpha*, determined a concentration-dependent inhibition of the contractile responses elicited by different agonists in guinea-pig ileum, with noncompetitive profile of inhibition curves in all cases. The mean CI_{50} values (and 50% confidence limit) calculated were: 247.08 (222.31/256.73) μg mL^{-1} for acetylcholine (Fig. 1), 818.14 (749.01/856.12) μg mL^{-1} for histamine (Fig. 2), 450.44 (415.37/462.13) μg mL^{-1} for serotonin (Fig. 3) and 210.09 (197.63/223.35) μg mL^{-1} for bradykinin (Fig. 4). The maximal inhibitions±SEM were: 83.13±8.23, 72.62±9.05, 75.82±5.17 and 97.35±5.37%, respectively. Although, the contractions of preparations were induced by different agonists, the inhibitory potency of hydroalcoholic extract of *G. polymorpha floccosa* did not significantly differ from agonist-induced contractions on the guinea pig ileum. As can be noted in all cases the extract

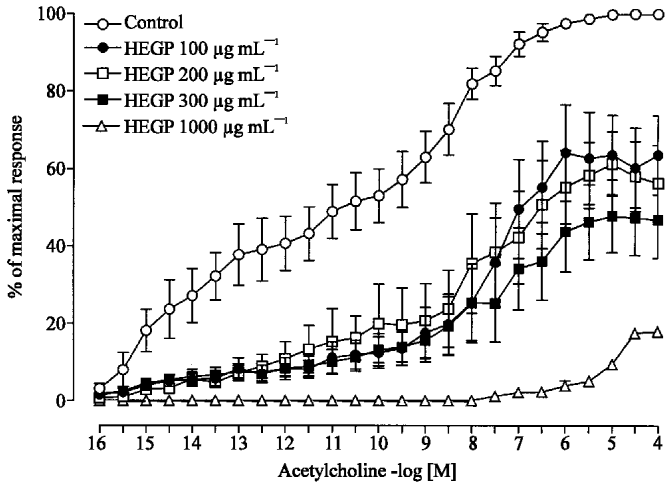

Fig. 1: Inhibitory effect of hydroalcoholic extract from *Gochnatia polymorpha floccosa* (HEGP) on acetylcholine-induced contractions in the isolated guinea-pig ileum. Each point represents the mean of 5-6 experiments and the vertical bars represent the SEM

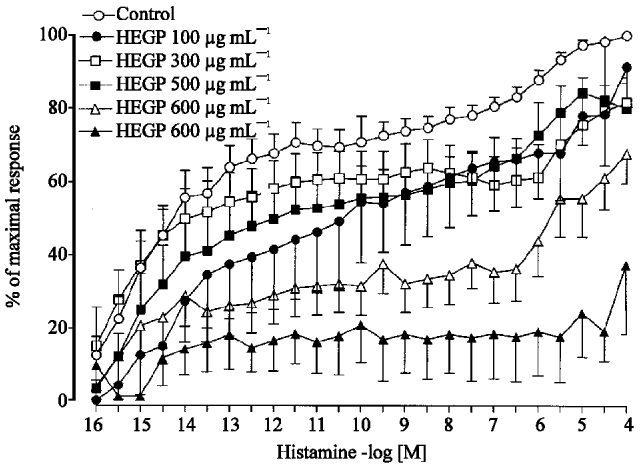

Fig. 2: Inhibitory effect of hydroalcoholic extract from *Gochnatia polymorpha floccosa* (HEGP) on histamine-induced contractions in the isolated guinea-pig ileum. Each point represents the mean of 5-6 experiments and the vertical bars represent the SEM

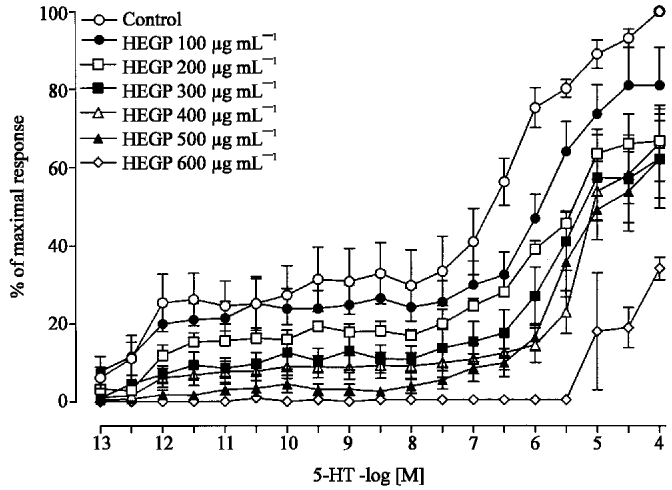

Fig. 3: Inhibitory effect of hydroalcoholic extract from *Gochnatia polymorpha floccosa* (HEGP) on serotonin-induced contractions in isolated guinea-pig ileum. Each point represents the mean of 6 experiments and the vertical bars represent the SEM

almost abolished the contractile responses. These results suggest that hydroalcoholic extract of *G. polymorpha floccosa* has inespecific antispasmodic activity for several contracturant agonists. The antispasmodic effect of *G. polymorpha floccosa* extract was reversible after successive washings of the preparations. The IC_{50}s for papaverine, used as a positive control drug, were 3.93 (2.28/4.14) µg mL^{-1}, for acetylcholine and 2.98 (1.87/4.92) µg mL^{-1}, for histamine (data not shown).

DISCUSSION

The experiments accomplished in isolated guinea-pig ileum with crude extract of leaves from *G. polymorpha*, indicates that the effect on intestinal smooth muscle is antispasmodic against

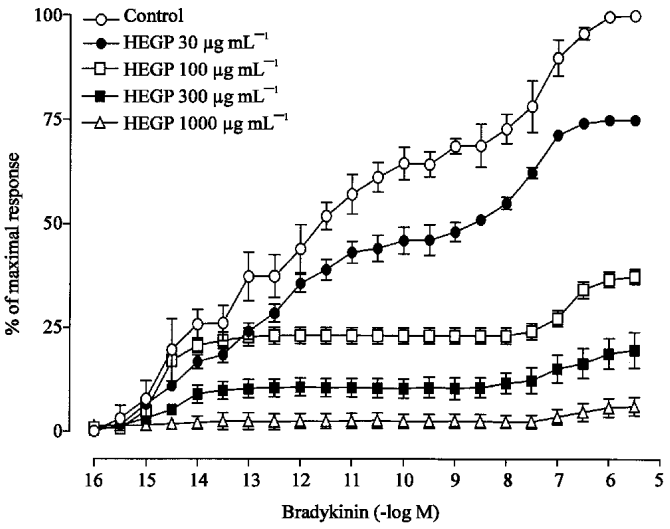

Fig. 4: Inhibitory effect of hydroalcoholic extract from *Gochnatia polymorpha floccosa* (HEGP) on bradykinin-induced contractions in isolated guinea-pig ileum. Each point represents the mean of 7 experiments and the vertical bars represent the SEM

acetylcholine, histamine, serotonin and bradykinin-induced contractions, which are excitatory neurotransmitters that exert physiologic control on gut motility (Campbell, 2009; Morini *et al.*, 1993; Chan and Rudd, 2006). Our current data show that this extract is also capable of inhibiting the response to a wide range of contractile stimuli, such as above neurotransmitters, although it shows no obvious selectivity among contractile agents.

Rare are the citation to concern the biological activity of chemical sub-products derived from *G. polymorpha*. The most significant scientific report is from Moreira *et al.* (2000), which revealed an antioedematogenic effects observed to isolated compounds obtained from the crude extract. For the first time, we shown in this paper an antispasmodic effects of hydroalcoholic extract of *G. polymorpha floccosa in vitro* and it was observed that a non specific inhibitory effect of the contractions induced by classical different contracting agonists, used in screening assay almost everyone and not permit us to conclude about the mechanisms involved with these antispasmodic effect observed in its experiments.

The result obtained with increasing concentration of extract of *G. polymorpha floccosa* (100 to 1000 μg mL^{-1}) after time incubation of twenty minutes, inhibited all agonists-induced contractions tested with a non-competitive profile of the concentration-response curves, but with significant potency and efficacy to a single extract, causing a full flattening of maximal response characterizing a non-competitive antagonism. The experimental model used by our group do not permit elucidate the precise mechanism by which the *G. polymorpha floccosa* extract exert antispasmodic effects. The only suggestion that our results can be drawn is that all contracturant agonists of this work use a common transduction signaling way to induce smooth muscle contraction (e.g., phospholipase C/inositol 1,4,5-triphosphate/diacylglycerol way, which is capable of mobilizing intracellular Ca^{2+}) (Hansen, 2003) and the *G. polymorpha floccosa* extract could interfere in any target site of this transduction way.

Its significant antispasmodic activity prompts as to carry out new future studies in order to confirm the effects described here using other *in vitro* experimental models, as well as, to isolate

new compounds from crude botanical material using polar and apolar fractions in order to obtain more potent antispasmodic agents. Phytochemical studies revealed in the plant stem a dehydrocostunolide lactone and the triterpene bauerenile (Farias *et al*., 1984). Aerial parts and roots supplied a larger amount of compounds of the bisabolene series and dimeric guaianolides (Bohlmann *et al*., 1986). Recent reports described the isolation of diterpenes, triterpenes, eudemanolide and flavonoids (Sacilotto *et al*., 1997).

Stefanello *et al*. (2006a) have found in the essential oil of flowers various phenylpropanoids, monoterpenes and sesquiterpenes. Also, Stefanello *et al*. (2006b) described a weak antimicrobial effects of three semi-purified fractions against gram positive bacteria and stronger activity with roots extract against gram positive bacteria and fungi (Stefanello *et al*., 2006b).

Consequently, it can be postulated that the antispasmodic activity exhibited by *G. polymorpha floccosa* extract must be due to the combined effects of several chemical constituents cited above and present in the plant, such as flavonoids, phenylpropanoids, or terpenes (Capasso *et al*., 2003; Sandraei *et al*., 2003; Liu *et al*., 2006) .

CONCLUSION

Finally, answers resulting confirm a new biological activity of leaves extract of *G. polymorpha floccosa* supporting and suggesting the popular use of this plant as a remedy to treat spasmogenic gastrointestinal diseases. Furthermore, new chemical and pharmacological approaches are necessary for determining of the biological effects of this plant, mainly on smooth muscle of the airways.

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REFERENCES

Bohlmann, F., C. Zdero, G. Schmeda-Hirschmann, J. Japucovic, X.A. Dominguez, R.M. King and H. Robinson, 1986. Dimeric guaianolides and other constituents from Gochnatia species. Phytochemistry, 25: 1175-1178.

Campbell, L., 2009. Gut motility and its control. Anaesth. Intens. Care Med., 10: 339-341.

Capasso, F., T.S. Gaginella, G. Grandolini and A.A.Izzo, 2003. Phytotherapy a Quick Reference to Herbal Medicine. Springer-Verlag, Berlin.

Chan, S.K. and J.A. Rudd, 2006. Role of bradykinin B_2 receptors in the modulation of the peristaltic reflex of the guinea pig isolated ileum. Eur. J. Pharmacol., 539: 108-115.

Chen, Y.F., H.Y. Tsai and T.S. Wu, 1995. Anti-inflammatory and analgesic activities from rots of angelica pubescens. Planta Med., 61: 2-8.

Correa, M.P., 1984. Dicionario das plantas uteis do Brasil e das exoticas cultivadas. Vol. 6 Ministerio da Agricultura, Instituto Brasileiro de Desenvolvimento Florestal, Imprensa Nacional, Rio de Janeiro.

Farias, A.C.M., A.J.R. Silva and T.C.B. Tomassini, 1984. Constituents of mochinea polymorpha. J. Nat. Prod., 47: 363-364.

Hansen, M., 2003. Neurohumoral control of gastrointestinal motility. Physiol. Res., 52: 1-30.

Jenkins, K.J., M. Hidirouglou and F.W. Collins, 1993. Influence of various flavonoids and simple phenolics on development of exudative diathesis in the chick. J. Agric. Food Chem., 41: 441-445.

Liu, Y., S. Zhanga and P.J.M. Abreu, 2006. Heterocyclic terpenes: linear furano and pyrroloterpenoids. Nat. Prod. Rep., 23: 630-651.

Lorenzi, H., 2002. Brazilian Trees. 4th Edn., Plantarum Institute, Nova Odessa.

Moreira, A.S., V. Spitzer, E.E.S. Schapoval and E.P. Schenkel, 2000. Antiinflam- matory activity of extracts and fractions from the leaves of *Gochnatia polymorpha*. Phytother. Res., 14: 638-640.

Morini G., J.F. Kuemmerle, M. Impicciatore, J.R. Grider and G.M. Makhlouf, 1993. Coexistence of histamine H1 and H2 receptors coupled to distinct signal transduction pathways in isolated intestinal muscle cells. J. Pharmacol. Exp. Ther., 264: 598-603.

Mors, W.B., C.T. Rizzini and N.A. Pereira, 2000. Medicinal Plants of Brazil. Reference Publications Inc., Michigan, ISBN: 0-917256-42-5, pp: 501.

Sacilotto, A.C.B.C., W. Vichnewski and W. Herz, 1997. Ent-kaurene diterpenes from *Gochnatia polymorpha* var. polymorpha. Phytochemistry, 44: 659-661.

Sandraei, H., G. Asghari and A.A. Hekmatti, 2003. Antispasmodic effect of three fractions of hydroalcoholic extract of *Pycnocycla spinosa*. J. Ethnopharmacol., 86: 187-190.

Schlemper, V., R. Medeiros, J. Ferreira, M.M. Medeiros and J.B. Calixto, 2005. Mechanisms underlying the relaxation response induced by bradykinin in the epithelium intact guinea pig trachea *in vitro*. Br. J. Pharmacol., 145: 740-750.

Simoes, C.M.O., E.P. Schenkel, G. Gosmann, J.C.P. De-Mello, L.A. Mentz and P.R. Petrovick, 2004. *Farmacognosy*: From Plant to Medicine. 5th Edn., UFRGS/UFSC., Porto Alegre.

Stark, D.M. and M.E. Ostrow, 1991. AALAS Training Manual Series. Vol. 1. Assistant Laboratory Animal Technician, Tennessee: Cordova TN, pp: 186.

Stefanello, M.E.A., A.C. Cervi, A. Wisniewski Junior and E.L. Simionatto, 2006a. O oleo essencial de *Gochnatia polymorpha* (Less) Cabr. ssp floccosa cabr. Quimica Nova, 29: 999-1002.

Stefanello, M.E.A., M.J. Salvador, I.Y. Ito and P.A.T. Macari, 2006b. Avaliacao da atividade antimicrobiana e citotoxica de extratos de *Gochnatia polymorpha* ssp floccosa. Braz. J. Pharmacognosia, 16: 525-530.

Wu, T.S., J.H. Yeh, M.J. Liu, H.Y. Tsai, Y.F. Chen and K.F.Huang, 1994. Antiinflammatory and analgesic principles from the roots of Angelica pubescens. Chin. Pharm. J., 46: 45-52.