

CHEMICAL AND PHARMACOLOGIC  
INVESTIGATION OF ALKALOIDAL EXTRACTS OF  
*BORRERIA VERTICILLATA*

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## ABSTRACT

### CHEMICAL AND PHARMACOLOGIC INVESTIGATION OF ALKALOIDAL EXTRACTS OF *BORRERIA VERTICILATA*

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*Borreria verticillata* is a member of the family Rubiaceae. It contains several alkaloids which are biologically active compounds. Decoctions of this herbaceous plant, locally called 'Alpha Marrow or Wild Margaret', are used in folklore practice as herbal medicine to lower blood pressure and as an abortifacient. Therefore the objectives of this study were to extract some of the alkaloidal fractions present, to investigate their pharmacological activities on the cardiovascular, uterine and gastrointestinal systems and simultaneously determine if there is any scientific rationale to these folklore practices.

Chemical analysis of *Borreria verticillata* resulted in the extraction of a primary or secondary alkaloidal fraction (BV1) and a quaternary alkaloidal fraction (BV2). Both alkaloidal extracts were used to carry out this investigation.

Rats were anaesthetized with 15% urethane (0.8 mL / 100 g body weight) and the femoral vein cannulated for intravenous administration of these alkaloidal extracts. The carotid artery was cannulated for blood pressure measurements that were recorded using a pressure transducer attached to a Grass polygraph. The heart was set up according to the

Langendorff's heart using the Harvard isolated heart perfusion apparatus and perfused with Krebs's Henslett physiological solution. Isotonic tensions were measured.

The potential activities of the alkaloidal extracts were also investigated using isolated uterine smooth muscle preparations, isolated thoracic aortic rings from mature Sprague-Dawley rats and isolated guinea-pig ileum smooth muscle. The isolated uterus, thoracic aorta and ileum were placed separately in a multi-channel organ bath system in DeJalon's solution for the uterus and Krebs's physiological solution for the thoracic aorta and guinea-pig ileum. The solutions were kept at 37 °C and aerated with CO<sub>2</sub>: O<sub>2</sub> (5%: 95%). Isometric tensions were measured using a FT3 force transducer connected to a Polyview.

On the rat blood pressure varying concentrations (3.89 mg/mL – 31.12 mg/mL BV1, 5.25 mg/mL – 41.84 mg/mL BV2) of both alkaloidal extracts showed a fall in blood pressure which was inhibited by atropine (0.05 µg/mL). Atropine is a known muscarinic receptor antagonist. The quaternary alkaloidal extract (BV2) not only produced a fall in blood pressure but at high concentrations ( $\geq 32$  mg/mL) produced a biphasic response (a fall then a significant rise in blood pressure). These results may be indicative of muscarinic activity.

Contractions of the thoracic aortic rings with intact endothelium were induced with phenylephrine (PE – 2.3 µg), a directly acting adrenomimetic amine and a systemic vasoconstrictor. Both alkaloidal fractions inhibited these contractions and hence showed dose response relaxation on the aortic rings. The muscarinic agonist acetylcholine (ACH) which was used comparatively on PE induced contractions on the thoracic aortic rings,

showed response similar to those of the extracts. The effects of these alkaloidal extracts and ACH were, however, blocked by (L-NAME – 0.02 µg), a nitric oxide antagonist.

Concentrations of 1.74 mg – 13.96 mg of BV1 produced dose dependent decrease in heart rate and contractile force. Similar results were seen with the use of 0.025 µg ACH. BV2 (3.75 mg – 31.36 mg), however, produced a dose dependent decrease in heart rate and increase in the force of contraction. The responses of BV2 were quite similar to those produced by 0.2 µg ACH (decrease in heart rate and an increase in force of contraction). Both alkaloidal extracts and ACH were antagonized by gallamine, a selective muscarinic, M<sub>2</sub>, antagonist.

BV2 (0.42 mg/mL – 3.35 mg/mL) produced dose dependent increases in the force of uterine contractions with sustained contractions at high doses of 3.33 mg / mL. The muscarinic agonist acetylcholine (ACH) (0.03 µg/mL – 0.27 µg/mL) was used comparatively and showed similar responses to BV2. The contractions produced by ACH and BV2 were antagonized by gallamine (0.05 µg/mL), a selective M<sub>2</sub> antagonist and indomethacin (1.0 mg / mL), a cyclooxygenase inhibitor. BV1 (0.12 mg/mL – 0.96 mg/mL), not only antagonized spontaneous uterine contractions but also inhibited dose dependent oxytocin-induced (0.3 µg/mL) contractions and contractions produced by BV2 and ACH on the uterus. BV2 (0.09 mg/mL – 0.73 mg/mL) contracted the ileum similarly to ACH and both contractions were antagonized by hexahydrosiladifenidol (HHSD – 0.23 µg/ mL), a selective M<sub>3</sub> antagonist. BV1 on the other hand also antagonized ACH-induced contractions and spontaneous contractions of the ileum.

The results showed that the fractions obtained have hypotensive effect which may be mediated through M2 muscarinic receptor on the heart, hence direct inhibition of cardiac activity and through the release of nitric oxide (endothelium derived relaxing factor – EDRF) from vascular endothelium. The release of nitric oxide led to relaxation of the vessels hence the fall in blood pressure. The results also suggested that *Borreria verticillata* contains an alkaloidal extract (BV2) which contracted the non-pregnant rat uterus and the guinea pig ileum through mediation of M2 and M3 muscarinic receptor subtypes respectively. The other alkaloidal extract BV1, inhibited the smooth muscle contractions in both the rat uterus and the ileum. BV1 is therefore acting as an antagonist of these muscarinic receptor subtypes.

The investigation showed that the alkaloidal extracts obtained from *Borreria verticillata* have biological activities and may therefore have therapeutic usefulness. It also showed the scientific rationale behind the folklore use of decoctions from this plant to lower blood pressure and as an abortifacient.

**Keywords:** *Borreria verticillata*; alkaloidal extracts (BV1 and BV2); acetylcholine (ACH – standard muscarinic agonist); muscarinic subtype receptors; cardiovascular, uterine, gastrointestinal systems.