Pharmacological basis for the folkloric uses of *Buxus wallichiana* in gastrointestinal, respiratory and vascular disorders
Introduction

*Buxus wallichiana* Baill. belonging to family Buxaceae, commonly known as Himalayan boxwood and shamas (Khare, 2007). Photochemical study revealed the presence of buxantine, buxiramin D, buxemol E, buwandrine F, buxamine F, buxaline H, (+)-16a, 31-diacetylbuxadine and semperviraminol (Kvaltinova et al., 1991; Husain et al., 1992; Rahman et al., 1999; Ata et al., 2002). Plant is traditionally used as laxative, diuretic, sedative, vermifuge, toothache, analgesic, anti-rheumatic, diaphoretic as well as used to treat hyper tension and bronchitis, asthma and airway congestion (Kritikar and Basu, 1989; Chopra et al., 1992; Fleming, 1999; Khare, 2007).

Despite the popular medicinal uses of *B. wallichiana*, no pharmacological data are available with respect to its usefulness in GIT motility, airway and vascular system disorders. This study was undertaken to investigate and validate the mechanisms mediating its medicinal uses in constipation, asthma, bronchitis and hypertension.

Material and Methods

Plant material and extraction

The wood of *B. wallichiana* was collected from Faisalabad, Pakistan in May, 2014 and identified by expert botanist at Botany Department, UAF, Faisalabad. The plant wood was freed from debris and adulterants by hand picking and grinded into coarse powder (#40) by herbal grinder.

Triple maceration procedure was adopted for extraction, by macerating powder with 80% aqueous-methanol in rotary orbital shaker, at room temperature.

Abstract

In *vivo* study was carried out to explore the pharmacological basis of crude extract of *Buxus wallichiana* for its folkloric uses in gastrointestinal, respiratory and vascular disorders. In jejunum preparations, crude extract (0.03 ± 1.0 mg/mL) caused a transient spasmogenic effect followed by the spasmolytic effect at higher doses (3.0–10 mg/mL). In atropinized jejunum preparation, crude extract inhibited the spontaneous and K+ (80 mM)-induced contraction, suggesting that spasmolytic effect is mediated through the Ca2+-channel blockade. The Ca2+-channel blockade effect was confirmed when pretreatment of tissue with extract produced a dose-dependent shift in Ca2+-concentration-response curves to the right, similarly as verapamil. Furthermore, crude extract exhibited non-specific relaxant effect on carbachol (1 µM) and K+ (80 mM)-induced tracheal contractions, suggesting the coexistence of anticholinergic and Ca2+-antagonistic properties. Moreover, it relaxed the K+ (80 mM)- and phenylephrine (1 µM)-induced contraction in rabbit aorta, suggesting the Ca2+-channel blockade. These findings may validate the folkloric uses of *B. wallichiana* in constipation, bronchitis, asthma and hypertension.
isolated rabbit jejunum segments (2 cm) suspended in tissue baths containing Tyrode's solution, aerated with carbogen (37°C). Stabilized rabbit jejunum preparations exhibit spontaneous rhythmic contractions and were tested for spasmogenic and/or spasmolytic effect without application of an agonist (Arshad et al., 2012; Janbaz et al., 2014a). The contractile effect of the plant material was assessed as the percent of the maximum effect produced by the control drug, acetylcholine (1.0 μM).

**Determination of Ca²⁺ channel blocking activity**
To assess whether the spasmolytic activity of the plant extract was through calcium channel blockade, the tissue preparation were depolarized by exposing to high concentration of KCl, i.e., K⁺ (80 mM), resulting in appearance of sustained contraction as previously described (Farre et al., 1991).

**Bronchodilator activity**
The bronchodilator effect of the plant extract was assessed by using isolated rabbit trachea preparations (2-3 mm width), suspended in tissue baths containing normal Kreb's solution (pH 7.4), aerated with carbogen (Gilani et al., 2005). Afterwards, stabilized tissue preparations were exposed to K⁺ (80 mM)- and carbachol (1 μM)-for stabilization with a dose interval of 45 min. The plant extract was applied on the obtained sustained contractions for possible relaxant effect. The standard drug with Ca²⁺ channel blocking effect (verapamil) was tested on K⁺ (80 mM)- and carbachol (1 μM)-induced spastic contractions in order to confirm the possible mechanism of action.

**Vasodilator activity**
The vasodilator/vasoconstrictor effect were studied by application of plant extract to tissue bath containing isolated rabbit thoracic aorta preparations (2-3 mm wide) in cumulative manner, already exposed to K⁺ (80 mM)- and phenylephrine (1 µM)- for stabilization with a dose interval of 45 min (Janbaz et al., 2014b).

**Data analysis and statistics**
The data is expressed as mean ± SEM and EC₅₀ (median effective concentration) values are given with 95% confidence intervals (95% CI) and the logarithmic dose response curves of different treatments were then plotted using computer software “Graphpad Prism” version 6, (Graph Pad Software, USA). Concentration-response curves were analyzed by nonlinear regression sigmoidal response curve (variable slope). Student t-test was applied for assessment of the observations. P<0.01 was believed to be statistically significant.

**Results**
Acute toxicity of B. wallichiana was tested at different doses (1, 3 and 5 g/kg); there was no mortality and...
change in animal behavior up to the dose as high as 5 g/kg, indicating that the plant is safe up to the maximal tested dose and is higher than the normal therapeutic dose.

Crude extract of *B. wallichiana* showed the spasmogenic effect on spontaneously contracting isolated rabbit jejunum preparations (0.03–1.0 mg/mL), which did not sustained and was subsequently followed by spasmodolytic effect at the next higher dose (3–10 mg/mL) with EC$_{50}$ of 2.87 mg/mL (95% CI: 2.12–3.35, n=3) (Figure 1). The observed contractile responses to plant extract was expressed as percentage of the maximal response to acetylcholine (0.3 µM), i.e. 15.50 ± 0.7, 21.17 ± 1.7, 25.30 ± 1.7, 39.3 ± 1.2 and 43.23 ± 5.2 (mean ± SEM, n=3), at the dose range of 0.01, 0.03, 0.1, 0.3 and 1.0 mg/mL respectively (Figure 2). In the presence of atropine (0.1 µM), spasmodenic effect was abolished while the spasmodolytic effect was observed with EC$_{50}$ of 1.17 mg/mL (95% CI: 0.42–1.95, n=3) (Figure 1).

When tested against K$^+$ (80 mM)-induced contractions, crude extract caused the dose dependent relaxation with EC$_{50}$ of 1.6 mg/mL (95% CI: 1.02–2.45, n=3), while verapamil, (positive control) inhibited the K$^+$ (80 mM)-induced contractions, with EC$_{50}$ of 0.17 mg/mL (95% CI: 0.09–0.35, n=3) (Figure 3). Crude extract shifted the Ca$^{2+}$-concentration response curves at the dose range of 0.3–3.0 mg/mL (n=3) to the right, like that caused by verapamil at 0.1–1.0 µM (n=3) (Figure 4).

Crude extract of *B. wallichiana*, on application to isolated rabbit tracheal preparations exhibited the concentration dependent relaxant effect on K$^+$ (80 mM)- and carbachol (1 µM)-induced contractions, with EC$_{50}$ value of 0.45 mg/mL (0.11–0.72, 95% CI, n = 3) and 1.13 mg/mL (0.63–1.62, 95% CI, n=3) respectively, being more potent against K$^+$ (80 mM)- (Figure 5A), whereas verapamil also exhibited the similar pattern of inhibition against K$^+$ (80 mM)- and carbachol (1 µM)-induced contractions with EC$_{50}$ value of 0.12 mg/mL (0.03–0.4,
95% CI, n = 3) and 1.12 mg/mL (0.59–1.94, 95% CI, n = 3) respectively (Figure 5B).

Crude extract of *B. wallichiana*, on application to isolated rabbit aorta preparations exhibited the concentration dependent relaxant effect on K+ (80 mM) and phenylephrine (1 µM)-induced contractions, with EC50 value of 0.28 mg/mL (0.07–0.63, 95% CI, n = 3) and 2.58 mg/mL (1.72–3.21, 95% CI, n=3) respectively (Figure 6A), whereas verapamil on application to the K+ (80 mM)- and phenylephrine (1 µM)-induced contractions showed relaxant effect with EC50 value of 0.15 mg/mL (0.02–0.42, 95% CI, n=3) and 1.25 mg/mL (0.62–2.11, 95% CI, n=3) respectively (Figure 6B). Crude extract shifted the Ca2+-concentration response curves at the dose range of 0.3–1.0 mg/mL (n=3) to the right, like that caused by verapamil at 0.1–0.3 µM (n=3), thus conforming that observed relaxant effect was likely to be mediated through calcium channel blocking effect (Figure 7 A & B).
Discussion

Medicinal plants are being widely used throughout the world as a source of potent medicinal agent for the health care. *B. wallichiana* has folkloric repute to be beneficial in the management of multiple ailments pertaining to gastrointestinal (constipation), respiratory (bronchitis, asthma and airway congestion) and vascular system (hypertension). Present research was undertaken for the scientific evaluation and validation of these folkloric claims through an exploration of the possible mechanism(s) of action.

Keeping in view of traditional uses, pharmacological study of crude extract of *B. wallichiana* was carried out on spontaneously contracting isolated rabbit jejunum preparations, to evaluate its possible effect, initially it produced the spasmogenic effect (contractile effect), which is usually mediated through cholinergic mechanism as like by acetylcholine (Gilani et al., 2005; Hussain et al., 2014), so for the conformation of this mechanism, spontaneous contracting jejunum preparations were pretreated with 0.1 µM of muscarinic receptor antagonist (atropine) (Delmendo et al., 1989), abolished the stimulatory effect of plant extract, which indicates that the *B. wallichiana* causes the gut stimulation via cholinergic pathway (Janbaz et al., 2012). Acetylcholine is a neurotransmitter released by the parasympathetic nervous system; regulate the peristaltic movement of the gut by acting on M3 muscarinic receptor while atropine (antagonist) blocks muscarinic receptors (Brown and Taylor, 1996). The observed spasmogenic effect of *B. wallichiana* validates its traditional use as laxative agent in the hypo-motility disorder of the gut, i.e., constipation. The spasmogenic effect was followed by the spasmyloytic effect at next higher doses of the extract, indicating the co-existence of spasmyloytic and spasmyloytic constituent(s), which is probably meant by the nature not to allow the spasmogenic effect going beyond the constipation, particularly at higher doses (Ghayur and Giliani, 2005).

In the previous studies, we observed that spasmyloytic effect of medicinal plants is usually mediated through

![Figure 6: Concentration-response curves showing inhibitory effect of (A) crude extract of *B. wallichiana* and (B) verapamil against K+ (80 mM)- and phenylephrine (1 µM)-induced contractions in isolated rabbit aortic preparations (values are expressed as mean ± SEM, n = 3)](image)

![Figure 7: Concentration–response curves of Ca2+ in the absence and presence of different concentrations of (A) crude extract of *B. wallichiana* and (B) verapamil in isolated rabbit aorta preparations (values are expressed as mean ± SEM, n = 3)](image)
Ca²⁺-channels blockade (Gilani et al., 1994; Janbaz et al., 2012). To elucidate whether the spasmylogetic effect of the extract is also mediated through the Ca²⁺ channels blockade, its extract was tested on K⁺ (80 mM)-induced contractions which is known to cause smooth muscles contractions through opening of the voltage dependent L-type Ca²⁺ channels, thus allowing the influx of extracellular Ca²⁺ causing a contractile effect (Bolton, 1979) and substances inhibiting the K⁺ (80 mM)-induced contraction are known as Ca²⁺ channels blockers, i.e., inhibitor of Ca²⁺ influx (Godfraind et al., 1986; Okumura et al., 1993; Shah et al., 2011). Crude extract of B. wallichiana, like verapamil (standard calcium channel blocker), relaxed the K⁺ (80 mM)-induced contractions, indicating the Ca²⁺ channel blocking action. The Ca²⁺ channels blocking effect of B. wallichiana was further confirmed when it shifted the Ca²⁺-concentration response curves to the right, like that caused by positive control (verapamil).

Based upon the traditional uses of B. wallichiana for the relief reparatory tract disorders including bronchitis, asthma and airway congestion, the plant extract was evaluated for its possible bronchodilator effect on sustained contractions induced on addition of carbachol (1 µM) and K⁺ (80 mM) to the tissue baths containing isolated rabbit trachea preparations and founded to produce relaxation in both of situations. The bronchodilator effect is likely to be mediated through Ca²⁺ channel blockade as Ca²⁺ channel blockers exert bronchodilator effect on hyperactive respiratory tract (Janbaz et al., 2014c).

Moreover, B. wallichiana exerted relaxant effect on isolated rabbit aortic preparations and also on K⁺ (80 mM)- and phenylephrine (1 µM)-induced contraction, elaborating the calcium channel blocking activity as K⁺ (80 mM)-induced contractions are mediated through the activation of Ca²⁺ channels as well as release of Ca²⁺ from endoplasmic reticulum, whereas mechanism underlying the phenylephrine (1 µM)-induced contraction is through the activation of α-adrenergic receptors and subsequent Ca²⁺ influx through the receptors mediated Ca²⁺ channels, hence suggesting its vasodilator effect possibly mediated through calcium channel blockade (Janbaz et al., 2014d).

Oral dose of B. wallichiana did not produced the lethality among the treated groups of mice up to tested dose of as high as 5 g/kg, which is much higher than the routinely used therapeutic dose. However, more detailed toxicity studies are required to justify the safety of this plant.

**Conclusion**

Gut modulatory (stimulitory and inhibitory) and bronchodilator activities of B. wallichiana may be attributed due to blockade of Ca²⁺ channels and muscarinic receptors, whereas, vasodilator activity may be due to blockade of Ca²⁺ channels, though additional mechanism cannot be ruled out.

**Financial Support**

Self-funded

**Conflict of Interest**

Authors declare no conflict of interest

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medicinal plants. Lucknow, Central Institute of Medicinal and Aromatic Plants Publication, 1992, p 89.


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