Tropical Journal of Pharmaceutical Research August 2017; 16 (8): 1865-1872
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v16i8.16

Original Research Article

Investigation of the laxative, spasmolytic and prokinetic properties of aqueous methanol extract of Buxus sempervirens Linn (Buxaceae)

Irfan Hamid* and Khalid Hussain Janbaz
Faculty of Pharmacy, Bahauddin Zakariya University, Multan (60000), Pakistan

For correspondence:* ***Email:* *Irfanhamid007@gmail.com;* ***Tel:*** *+92-3017470522*

Sent for review: 22 February 2017 *Revised accepted: 16 July 2017*

Abstract

Purpose: *To investigate the spasmolytic and laxative properties of Buxus sempervirens Linn (Buxaceae) in rabbits and mice.*
Methods: *Aqueous methanol extract (AqMeBS) as well as the dichloromethane (DCMF) and aqueous (AqF) fractions of Buxus sempervirens were investigated on isolated rabbit jejunum to explore its antispasmodic effect, relative to the standard drug, verapamil. Laxative and prokinetic potentials of 250 and 500 mg/kg doses of AqMeBS extract were evaluated in mice and compared to that of negative (normal saline) and positive (carbachol) control groups. The effects of AqMeBS and carbachol were also tested in mice pretreated with atropine (10 mg/kg). Single dose, acute oral toxicity study on AqMeBS was also executed in mice at 4000, 8000 and 12000 mg/kg doses.*
Results: *AqMeBS, DCMF and AqF significantly inhibited the rhythmic contractility of jejunum with 0.961, 0.0327 and 0.242 mg/mL, respectively, as median effective concentrations (EC_{50}). In addition, AqMeBS, DCMF and AqF significantly relaxed the contractions due to K^+, with EC_{50} of 1.85, 0.05 and 1.07 mg/mL, respectively. Ca^{2+} concentration response curves (CCRCs) were shifted to the right by AqMeBS and DCMF, in the same manner as verapamil. In the in vivo experiments, AqMeBS produced significant ($p < 0.0001$) laxative and prokinetic effects at 250 and 500 mg/kg doses and was comparable to that of carbachol. The acute toxicity study showed that AqMeBS was associated with one mortality at the highest tested dose (12000 mg/kg).*
Conclusion: *These results provide the pharmacological basis for the traditional use of B. sempervirens Linn as a laxative and prokinetic remedy in the management of constipation.*

Keywords: *Buxus sempervirens, Calcium channel blocker, Prokinetic, Laxative, Spasmolytic*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Buxus sempervirens known in Pakistan as *paapari* and is an evergreen tree widespread, especially in the Swat area of Pakistan. This Himlalayas plant is characterized by its elliptic-shaped leaves and tetragonal shoots [1]. The evergreen plants of *B. sempervirens* are popularly used for the decorative purpose. The plant is used in folkloric medicine for the treatment of alopecia, neoplasm, seizures, liver diseases (Hepatitis), malaria, pyrexia, pneumonia, skin irritation, arthritis and stomatitis. It is also used as an astringent, cardiac stimulant and purgative. It is also used for the management of constipation, skin disorders, hyperuricemia, hypertension, immobility, tetanus and infections caused by mycobacterium [2]. Several alkaloids e.g. semperviraminol, buxamine F, 17-oxocycloprotobuxine, buxoxy-

benzamine, buxapapillinine and cyclobuxine-D have been isolated from *B. sempervirens* [3]. The plant also exhibits several pharmacological activities such as anticancer [4], anti-protozoal, antioxidant, anti-malarial, anti-cholinesterase and tyrosinase- inhibitory effects [5].

The objective of present study was to investigate the spasmolytic, prokinetic and laxative potential of the plant in mice and rabbit models.

EXPERIMENTAL

Chemicals and drugs

Ethylene-diamine-tetra-acetic acid (EDTA), potassium chloride (KCl), carbachol, atropine and acetylcholine were products of Sigma®. All other chemicals were also of analytical grade and acquired from BDH® and Merck®.

Preparation of crude extract and fractions

Fresh aerial parts of *B. sempervirens* were from the Swat valley in 2014. Authentication of the plant was done in University of the Punjab, Lahore by a taxonomist Dr. Niazi, (who assigned a voucher no. 252014) and the sample was submitted to the departmental herbarium. The aerial portion was dried and pulverised. The powdered sample was then extracted in aqueous-methanol (30:70 volume ratio of water : methanol) to produce an extract yield of 10.075 %. The extract (AqMeBS, 50 g) was then dissolved in enough distilled water and an equal volume of dichloromethane (DCM) was added to it. The extraction was repeated thrice, and the resultant extracts were combined and concentrated with a rotary evaporator (model 9230, BUCHI Switzerland) at 37 °C. The aqueous part was lyophilized to yield AqF. The yield of AqF was 21.6 %, while that of DCMF was 78.4 % [6].

Phytochemical screening

Phytochemical evaluation of AqMeBS was performed according to the standard methods described earlier [7].

Experimental animal conditions

Mature, local breed of rabbits (mean weight = 1.5 kg) and albino mice (Swiss breed, weight: 20 - 25 g) of both sexes were procured from a supplier in Multan. The rabbits and mice were housed under standardized conditions at 25 ± 1 °C and provided unrestricted access to balanced diet (Hi-Tech Feeds Pvt. Ltd. Lahore) and clean drinking water. The animals were fasted overnight prior to commencement of the study. The rabbits were anesthetized with chloroform and sacrificed by cervical dislocation to excise jejunum for use in *in vitro* experimentations. All experimental procedures were according to the directives of the Institute of Laboratory Animal Resources, Commission on Life Sciences [8]. Approval (EC/05PhDL/2013) was obtained from the Animal Ethics Committee of Bahauddin Zakariya University, Multan.

Evaluation of *in vitro* anti-spasmodic effect

The anti-spasmodic activities of AqMeBS, DCMF and AqF were investigated in isolated rabbit jejunum preparation. A 2-3 cm portion of the jejunum was placed in an organ bath containing 15 mL Tyrode's solution. The composition of Tyrode's solution (mM) was: sodium chloride (136.9), KCl (2.68), sodium bicarbonate (11.90), magnesium chloride (1.05), sodium dihydrogen phosphate (0.42), calcium chloride (1.8) and glucose (5.55). The tissue was kept at normal body temperature and aerated using carbogen gas (95 % oxygen and 5 % carbon dioxide). Each jejenum tissue was subjected to pre-load of 1g and allowed to stabilize for half an hour. Then doses of test material were administered in a cumulative manner after every 10 min. A rhythmic contractility was detected in each jejunum preparation which allowed the assessment of relaxing potential of the extracts (0.03-3 mg/mL AqMeBS; 0.03-0.1 mg/mL DCMF and 0.03-3 mg/mL AqF) in the absence of agonist. Isotonic transducers were used to record the rhythmic contractions via a Power Lab (AD Instruments, Australia) [9].

Determination of *in vitro* calcium channel blocking effect

The aqueous methanolic extract (AqMeBS) and its dichloromethane fraction (DCMF) were further investigated to ascertain the mechanism underlying their effects. On exposure to 80mM K^+, the isolated jejunum produced sustained contractility through voltage-dependent calcium channels (VDCs) [10]. Isolated jejunum preparations were subjected to the solutions of test extracts which exerted concentration dependent inhibition in contractility. This inhibition in contractility was compared to that of verapamil, a calcium channel blocker. The inhibition in contractility with calcium channel blocker was validated in isolated jejunum. The isolated jejunum was steadied in Tyrode's solution and was then substituted with calcium free Tyrode's solution (comprising of the constituents as that of Tyrode solution, however, lacks in calcium chloride). Thereafter, the

calcium free Tyrode's solution was replaced with EDTA-rich, Ca^{2+}-free Tyrode's solution (comprising of the same constituents as that of calcium free Tyrode solution, however, contains EDTA, 0.1 mM). Calcium chloride (0.01 and 0.1 mM) was added to the organ bath after 30 minutes, in accumulative manner to attain control Ca^{2+} concentration response curves (CRCs). The trial was repeated multiple times until alike Ca^{2+} control CRCs were obtained. The tissues were then rinsed and equilibrated with AqMeBS and DCMF for 45 mins. Thereafter, Ca^{2+} CRCs produced in response to varying dilutions of AqMeBS, DCMF and verapamil were constructed and matched with control CRCs [11].

Determination of laxative effect in albino mice

Seven groups of mice were used (n=5). Mice in negative control group were orally administered with 10 ml/Kg normal saline. Mice in positive control Group were given the laxative, cabachol (1 mg/kg) through intraperitoneal route. Doses of 250 and 500 mg/ Kg AqMeBS were administered to the mice in group 3 and 4 respectively. Mice, pretreated with intraperitoneal atropine (10 mg/Kg) were subdivided into group 5, 6 and 7 that subsequently received 1 mg/Kg carbachol and 250 and 500 mg/Kg AqMeBS extract respectively. The total number of droppings and total number of moist droppings in all the groups were recorded after 6 hours. The increase in moist droppings comparative to the total droppings was an indicative of laxative effect [12].

Evaluation of gastrointestinal motility

The mice were randomly divided into seven groups. These were kept overnight with an access to water, however, were devoid of any food. In group 1 (negative control), 1 mL normal saline was given to the mice *p.o*. Group II mice (positive control) received 1 mg/kg CCh *i.p.,* while mice in groups 3 and 4 received 250 and 500 mg /Kg of AqMeBS (*p.o*), respectively. The acetylcholine-like prokinetic properties of carbachol and AqMeBS were also studied. Mice in groups 5, 6 and 7 received 10 mg/kg atropine *i.p* 15 min before treatment with carbachol (1 mg/Kg) and AqMeBS (250 and 500mg/Kg) respectively. 30 minutes post administration of initial therapy, each mouse also received 1 mL (5 % w/v) charcoal suspension consisting of (0.5 %w/v) methyl cellulose. These were anesthetized with chloroform and sacrificed 30 minutes after the charcoal meal. A percentage of intestinal propulsion (an index of distance moved by the charcoal) was calculated as outlined earlier [13].

% Intestinal propulsion = Distance traveled by charcoal diet (cm)/total length of small intestine (cm) ×100

Acute toxicity Study

Four groups of mice were used (n=5, one group per cage). The acute toxicity study was carried out at 25 ± 01 °C and 12 hour in a well-aerated room with 12 h/12 h day/night cycle. The mice were fasted overnight however, had free access to water. Mice in group 2, 3 and 4 were given a single oral dose of 4000, 8000 and 12000 mg/Kg respectively, while, mice in group 1 received 10 ml/Kg normal saline orally. Mice in all the groups were monitored non-stop for the first 48 h, for toxicity signs such as mortality, behavioral changes and weight changes. The monitoring continued for 14 days [14]. All observed toxicity signs were recorded.

Statistical analysis

Data from laxative and prokinetic studies were analyzed with one-way ANOVA using Graph Pad Prism® version 6 (San Diego, California USA). $P < 0.0001$ was taken as indicative of statistically significant differences. Data from *in-vitro* experiments were shown as mean ± Standard error of mean (SEM). EC_{50} was determined from the logarithmic dose response curves with GraphPad Prism®.

RESULTS

Phytochemical profile

The results of phytochemical investigations are presented in Table 1.

Table 1: Phytochemical profile of *Buxus sempervirens* Linn extract

Secondary metabolites	Result
Alkaloids	Present
Cardiotonic glycosides	Present
Saponins	Present
Tannins	Present
Flavonoids	Present
Coumarins	Present

Assessment of *in-vitro* antispasmodic effect

AqMeBS induced spasmodic activity at 0.1-1 mg/mL dose, which was followed by anti-spasmodic action at 3 mg/mL dose. The spasmodic effect was entirely blocked by pre-treatment with 3 µM atropine (Figure 1).

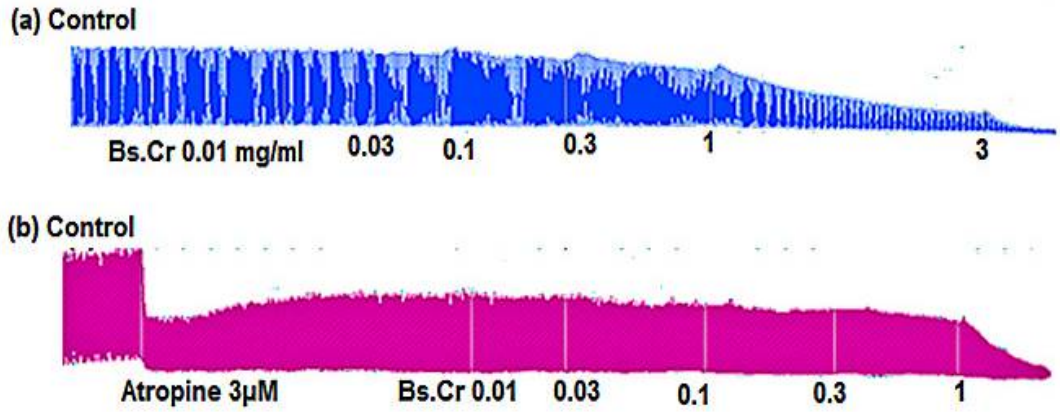

Figure 1: Anti-spasmodic effect of AqMeBS in the absence and presence of atropine.

AqMeBS and AqF relaxed the rabbit jejunum contractions at doses of 0.01 - 0.1 mg/mL, showed 0.032 mg/mL EC_{50} (95 % CI = 0.022 - 0.04) while 0.01 - 3 mg/mL showed EC_{50} = 0.24 mg/mL (95 % CI = 0.15 - 0.38), respectively.

AqMeBS, DCMF and AqF relaxed dose-dependently, the potassium (80 mM)-induced contraction with EC_{50} values of 1.85 mg / mL (95% CI = 1.5 - 2.1), 0.05 mg / mL (95% CI = 0.03 - 0.09), and 1.07 mg / mL (95% CI = 0.75 - 1.5), respectively. The corresponding EC_{50} for verapamil was 0.82 mg / mL (95% CI = 0.82 to 0.82). These results are shown in Figure 2.

In vitro calcium channel blocking effect

AqMeBS and DCMF shifted the CRCs to the right at 0.3 – 3 mg/mL and 0.03 - 0.3 mg/mL, respectively, in a manner similar to the shift produced by 0.1 - 1µM verapamil (Figure 3).

Laxative effect

A high number of wet droppings resulted from administering 250 and 500 mg/Kg doses of AqMeBS [51.67 ± 3.73 % (***p < 0.001) and 64.70 ± 2.59 % (****p < 0.0001)], respectively. However, carbachol administration resulted in the highest wet droppings [73.9 ± 3.85 % (****p < 0.0001)]. In comparison, prior administration of atropine resulted in significant decreases in the amount of wet droppings in the AqMeBS group. The atropine-induced reduction in wet droppings was most pronounced in the CCh group [9 ± 5.18 % (****p < 0.0001)]. These results are shown on Table 2.

Effect of extracts on intestinal transit

Prokinetic effect was produced by AqMeBS at 250 and 500 mg/kg as evidenced by the intestinal movement of the charcoal meal. The charcoal meal traveled the least distance in the group given saline (56.6 ± 0.4 %). Intestinal transit was significantly increased by CCh (92.2 ± 1.11 %, p < 0.0001). 250mg/kg dose of AqMeBS produced 64 ± 0.32 % (p < 0.001) whereas, 500mg/Kg dose of AqMeBS produced 77.4 ± 0.6 % intestinal propulsions (p < 0.0001). However, the prokinetic activities of AqMeBS and carbachol were significantly attenuated by prior treatment with 10 mg/kg atropine. These results are presented in Figure 4.

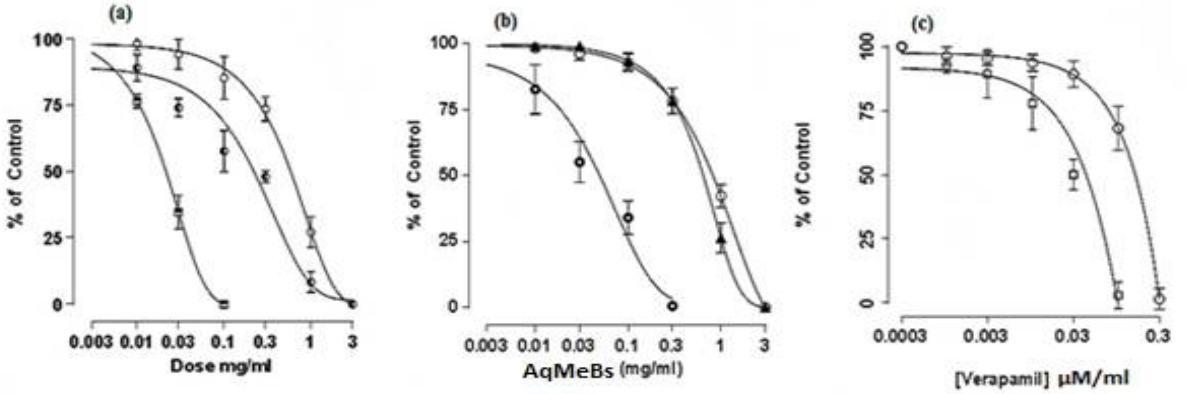

Figure 2: Relaxant effect of AqMeBS, DCMF, AqF and verapamil in rabbit jejunum. **(a)** Sponteneous**:** AqMeBS (○), AqF (◐), DCMF (▬); **(b)** Against pottasium (80 mM)-induced contraction**:** AqMeBS (○) AqF (▲) DCMF (●); **(c)** Verapamil: sponteneous (○), K^+ (80 mM)-induced contraction (□)

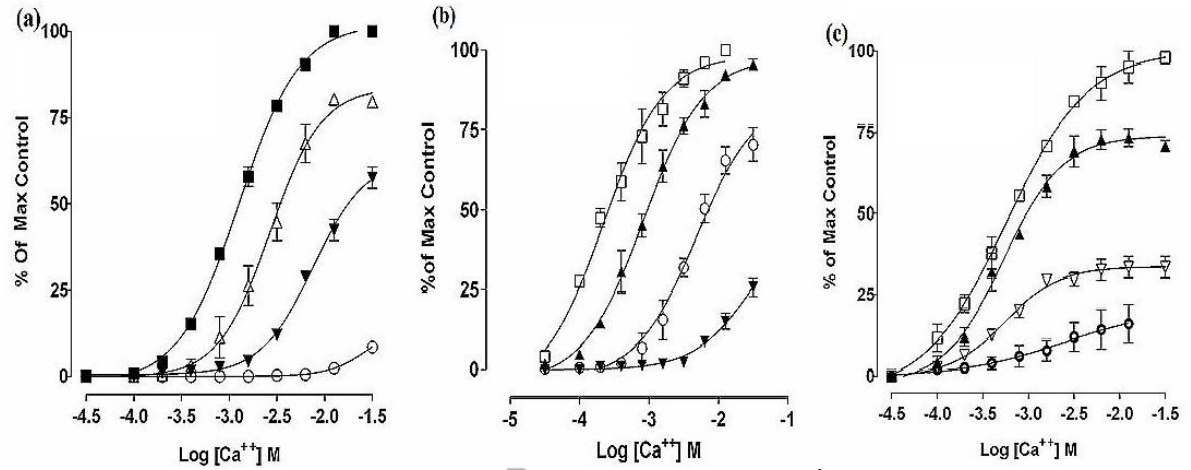

Figure 3: Effect of **(a)** AqMeBS: control (■), 0.3 mg/mL I mg/mL (△) (▼), 3mg/mL (○); **(b)** DCMF: control (□), 0.03mg/mL (▲) 0.1 mg/mL (○), 0.3 mg / mL (▼); **(C)** Verapamil: control (□), 0.03 µM/mL (▲), 0.1 µM / mL (▽) and 0.03mg/mL (⊙) on CRCs of rabbit jejunum.

Table 2: Laxative property of AqMeBS

Treatment	Mean defecation/ group	Mean no of wet feces/ group	Mean wet feces (%)
Normal saline p.o, (10 mL/kg)	3.4 ± 0.24	0	0
Carbachol intraperitoneal (1 mg/kg).	13.8 ± 0.37****	10.2 ± 0.37****	73.9 ± 3.85
AqMeBS p.o, (250 mg/kg)	6.2 ± 0.37****	3.2 ± 0.2***	51.67 ± 3.73
AqMeBS p.o, (500 mg/kg)	11.6 ± 0.24****	7.5 ± 0.15****	64.70 ± 2.59
CCh intraperitoneal (1mg/kg) + atropine intraperitoneal (10mg/kg)	4.4 ± 0.24****	0.4 ± 0.1****	9 ± 5.18
AqMeBS p.o, (250mg/kg) + atropine intraperitoneal (10 mg/kg)	4.7 ± 0.2**	1.6 ± 0.1***	34.17 ± 4.49
AqMeBS p.o, (500mg/kg) + atropine intraperitoneal (10mg/kg, i.p.)	4.2 ± 0.37****	0.7 ± 0.12****	16.33 ± 3.75

Note: * showed the significance in comparison of group 2, 3 and 4 against control group 1 at $p < 0.05$; ** showed the significance in comparison of group 5 against carbachol group at $p < 0.01$; *** showed the significance in comparison of groups 2, 3 and 4 against control group 1 at $p < 0.001$; **** showed the significance in comparison of group 6 against 250 mg/kg AqMeBS group and group 7 against 500 mg/kg AqMeBS group at $p < 0.0001$. Data were expressed as mean ± SEM (n = 5); p.o. = per oral

Acute toxicity

No signs or symptoms of toxic effects were seen in the mice treated with AqMeBS at a dose of 4000 mg /kg. However, minor toxicity signs were noticed in the groups that received doses of 8000 and 12000 mg/kg, although these signs receded by the 14th day of the acute toxicity test (Table 3).

DISCUSSION

The traditional use of *B. sempervirens* as a laxative and spasmolytic [2,15] formed the bases The mechanism underlying the laxative potential of AqMeBS in mice was revealed by the concentration-dependent, extract-induced contractility of rabbit jejenum, which was inhibited by atropine. The increase in jejenum contractility might be due to the stimulatory influence of some phytochemical components of *Buxus sempervirens* on intestinal muscarinic receptor of this study. Results from phtochemical analysis showed that AqMeBS contains flavonoids, tannins, alkaloids and saponins. These phytochemicals may be responsible for the traditional and medicinal application of this plant [16]. This study has demonstrated that *B. sempervirens* possesses antispasmodic and prokinetic activities. Similar results were obtained in studies on *Buxus wallichiana* (a plant in the same genus) which is used for treating gastrointestinal, cardiovascular and respiratory illnesses [15].

[17]. The candidate compounds are probably more abundant in DCMF, which inhibited jejunum contractility most. It has been suggested that the spasmolytic properties of herbal extracts may be a consequence of inhibition of calcium channels [18]. Studies have established unequivocally that potassium ions open voltage-gated calcium channels, a process that triggers influx of

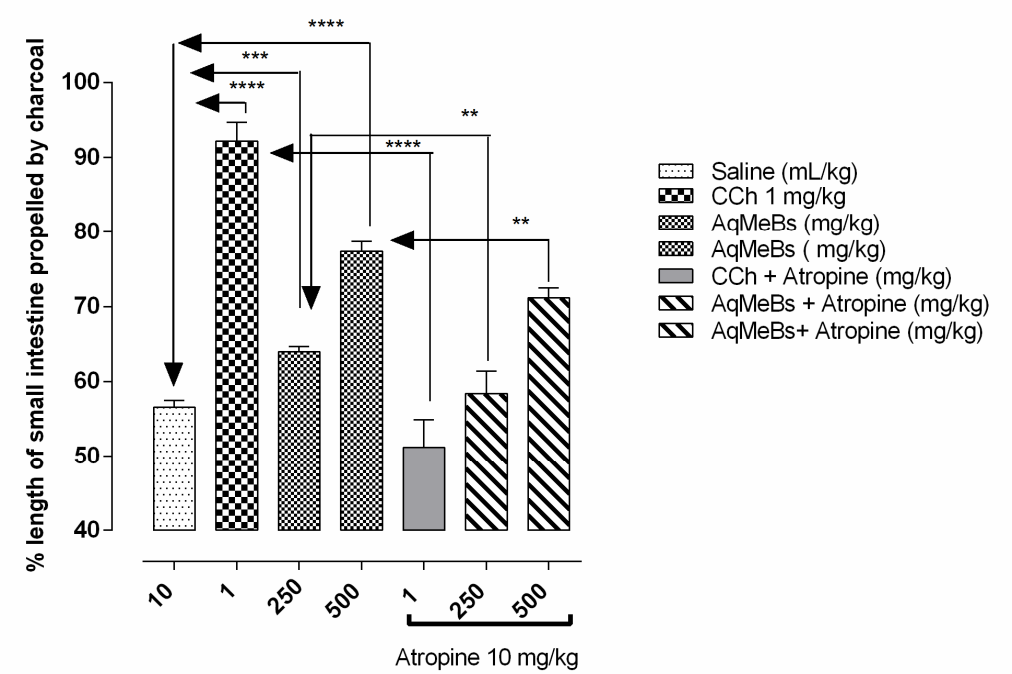

Figure 4: Effect of AqMeBS on gastrointestinal motility of charcoal meal, with or without atropine. * $p < 0.05$, ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. Results are expressed as mean ± SEM (n = 5)

Table 3: Oral acute toxicity of AqMeBS

Treatment dose (mg/kg)	Mice	Mortality	Signs of toxicity
Contro l (normal saline, 10 ml/kg)	5	0	None
4000 mg/kg AqMeBS	5	0	None
8000 mg/kg AqMeBS	5	0	No sign or symptom of toxicity were witnessed within six hours of dosing. On second day, ruffled skin, piloerection, more defeces and increased urination were observed.
12000 mg/kg AqMeBS	5	1 at day six	Decreased physical activity, depressed mood, piloerection, anal swelling and bleeding were witnessed four hours after administration

extracellular Ca^{2+} resulting in enhancement of smooth muscle contractility [19]. The effect of AqMeBS was evident from the shifting of the CRCs to right, an effect resembling that of the calcium channel blocker, verapamil. More potent effects were produced by DCMF and verapamil than AqMeBS fraction. This is consistent with the superior jejenum contractile responses seen in this fraction, relative to AqMeBS. Again, this effect suggests that DCMF contains more potent calcium channel-blocking agents than AqMeBS.

The results acquired in this study showed that AqMeBS possesses laxative properties comparable to that of carbachol, an agent which enhances motility of the small intestine [20]. The laxative effect of AqMeBS was inhibited partially by atropine, an established antagonist of muscarinic receptor. Atropine blocks cholinergic nicotinic receptor in the intestine where the release of acetylcholine from myenteric plexus activates cholinergic receptors [21]. The inhibition of the laxative effect of AqMeBS by atropine suggests that the extract may contain compounds that exert the same effects as acetylcholine. Acetlycholine increases gastrointestinal contraction by stimulating muscarinic receptors in a process mediated by G-protein, phospholipase C and inositol triphosphate [22]. Previous studies have also reported that *B. sempervirens* extract inhibited the activities of butrylcholinesterase and acetylcholinesterase [23]. These findings strongly suggest that some components of AqMeBS may have cholinergic properties, which are responsible for blockage of the activity of acetylcholinesterase [24]. Indeed, some parasympathomimetic alkaloids and tannins have been reported in *B. sempervirens* [16]. Thus the presence of acetylcholine-like components may be responsible for the cholinergic activity of *B. sempervirens* [25].

Results from oral acute toxicity study in mice revealed that the lethal dose for 50 percent animals was more than 12000 mg/kg. The weight loss recorded may be due to the saponins in the extract. It has been reported that saponins may be implicated in loss of weight in some experimental animals [26].

CONCLUSION

The findings of this study show that *B. sempervirens Linn* exhibit laxative, prokinetic and antispasmodic potential. These results may lend some pharmacological justification for the traditional use of the plant in constipation and other gastrointestinal problems.

DECLARATIONS

Acknowledgement

The authors wish to thank to the Chairman, Department of Pharmacy, B.Z.U., Multan, Pakistan for providing all research facilities and animals for this study.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

REFERENCES

1. *Khare CP. Indian medicinal plants: an illustrated dictionary. Berlin, Germany: Springer-Verlag; 2007; p 106.*
2. *Duke JA. Handbook of phytochemical constituents of GRAS herbs and other economical plants. London: CRC Press; 1992; pp 115-116.*
3. *Ata A, Naz S, Choudhary MI, Sener B, Turkoz S. New Steroidal Alkaloids from the Roots of Buxus sempervirens. J Nat Prod 1999; 62(5): 665-669.*
4. *Ait-Mohamed O, Battisti V, Joliot V, Fritsch L, Pontis J, Medjkane S, Redeuilh C, Lamouri A, Fahy C, Rholam M, et al. Acetonic extract of Buxus sempervirens induces cell cycle arrest, apoptosis and autophagy in breast cancer cells. PLoS One 2011; 6(9): e24537.*
5. *Orhan IE, Erdem SA, Senol FS, Kartal M, Sener B. Exploration of cholinesterase and tyrosinase inhibitory, antiprotozoal and antioxidant effects of Buxus sempervirens L. (boxwood). Ind Crops Prod 2012; 40: 116-121.*
6. *Saqib F, Janbaz KH. Rationalizing ethnopharmacological uses of Alternanthera sessilis: A folk medicinal plant of Pakistan to manage diarrhea, asthma and hypertension. J Ethnopharmacol 2016; 182: 110-121.*
7. *Saeed N, Khan MR, Shabbir M. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts Torilis leptophylla L. BMC Complement Altern Med 2012; 12(1): 221.*
8. *Care A, Use Committee. Guidelines for the capture, handling, and care of mammals as approved by the American Society of Mammalogists. J Mammal 1998; 1: 1416-1431.*
9. *Janbaz KH, Hamid I, Gilani A-u-H, Qadir MI. Spasmolytic, Bronchodilator and Vasodilator Activities of Aqueous-methanolic Extract of Ocimum basilicum. Int J Agric Biol 2014; 16(2): 321-327.*
10. *Mehmood MH, Siddiqi HS, Gilani AH. The antidiarrheal and spasmolytic activities of Phyllanthus emblica are mediated through dual blockade of muscarinic receptors and Ca2+ channels. J Ethnopharmacol 2011;133(2):856-865.*
11. *Janbaz KH, Arif J, Saqib F, Imran I, Ashraf M, Zia-Ul-Haq M, Jaafar HZ, Feo VD. In-vitro and in-vivo validation of ethnopharmacological uses of methanol extract of Isodon rugosus Wall. ex Benth.(Lamiaceae). BMC Complement Altern Med 2014; 14(1): 71.*
12. *Muhammad N, Rehman N, Khan H, Saeed M, Gilani AH. Prokinetic and laxative effects of the crude methanolic extract of Viola betonicifolia whole plant in rodents. BMC Complement Altern Med 2013; 13(1): 70.*
13. *Ghayur MN, Gilani AH. Pharmacological basis for the medicinal use of ginger in gastrointestinal disorders. Dig Dis Sci 2005; 50(10): 1889-1897.*
14. *Rhiouani H, El-Hilaly J, Israili ZH, Lyoussi B. Acute and sub-chronic toxicity of an aqueous extract of the leaves of Herniaria glabra in rodents. J Ethnopharmacol 2008; 118(3): 378-386.*
15. *Hussain M, Raza SM, Janbaz KH. Pharmacological basis for the folkloric uses of Buxus wallichiana in gastrointestinal, respiratory and vascular disorders. Bangladesh J Pharmacol 2015; 10(2): 260-266.*
16. *Wink M. Modes of action of herbal medicines and plant secondary metabolites. Medicines 2015; 2(3): 251-286.*
17. *Khan A-u, Rahim A, Iqbal Z, Gilani A-H. Insights into mechanisms underlying the gut and airways modulatory*

effects of Swertia chirata. J Nat Med 2012; 66(1): 140-148.

18. *Shah AJ, Gilani AH. The calcium channel blocking and phosphodiesterase inhibitory activities of the extract of Andropogon muricatus explains its medicinal use in airways disorders. Phytother Res 2012; 26(8): 1256-1258.*
19. *Godfraind T, Miller R, Wibo M. Calcium antagonism and calcium entry blockade. Pharmacol Rev. 1986; 38: 321-326.*
20. *Gilani AH, Ghayur MN, Saify ZS, Ahmed SP, Choudhary MI, Khalid A. Presence of cholinomimetic and acetylcholinesterase inhibitory constituents in betel nut. Life Sci 2004; 75(20): 2377-2389.*
21. *Tobin G, Giglio D, Lundgren O. Muscarinic receptor subtypes in the alimentary tract. J Physiol Pharmacol 2009; 60(1): 3-21.*
22. *Mandl P, Kiss Role of presynaptic nicotinic acetylcholine receptors in the regulation of gastrointestinal motility. Brain Res Bull 2007; 72(4): 194-200.*
23. *Orhan I, Şener B, Choudhary M, Khalid A. Acetylcholinesterase and butyrylcholinesterase inhibitory activity of some Turkish medicinal plants. J Ethnopharmacol 2004; 91(1): 57-60.*
24. *Caulfield MP, Birdsall NJ. International Union of Pharmacology. XVII. Classification of muscarinic acetylcholine receptors. Pharmacol Rev 1998; 50(2): 279-290.*
25. *Gilani AH, Bashir S, Janbaz KH, Shah AJ. Presence of cholinergic and calcium channel blocking activities explains the traditional use of Hibiscus rosasinensis in constipation and diarrhoea. J Ethnopharmacol 2005; 102(2): 289-294.*
26. *Wang L, Li Z, Li L, Li Y, Yu M, Zhou Y, Zhou Y, Lv X, Arai H, Xu Y. Acute and sub-chronic oral toxicity profiles of the aqueous extract of Cortex Dictamni in mice and rats. J Ethnopharmacol 2014; 158: 207-215.*