Anthelmintic and Relaxant Activity of *Verbascum Thapsus* Mullein.

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Abstract

Background

*Verbascum thapsus* Mullein belongs to a family, “Scrophulariaceae” that has many traditional uses as antispasmodic, expectorant, antitubercular, antiasthmatic, and wormicidal. The present research describes the scientific rationale for ethnobotanical anthelmintic activity of crude extract of *Verbascum thapsus*. Traditional use of *Verbascum thapsus*, in abdominal pain as relaxant (antispasmodic activity) is also explored. Preliminary phytochemical analysis was performed, too.

Methods

Aqueous methanolic extract of *Verbascum thapsus* (Vt.Cr) was prepared by soaking the shade dried choked aerial parts of *Verbascum thapsus* in commercial grade methanol. Preliminary, phytochemical screenings were performed. Dried extract was tested for anthelmintic activity on round worms (*Ascaridia galli*) and tape worms (*Raillietina spiralis*) as per reported procedures. Separate groups, carrying six matured test worms of equal lengths, denoted as negative control and positive control with albendazole (10 mg/ml), and Vt.Cr at concentrations 10, 20, 40 mg/ml (n=6) were selected for the assay. Time taken for paralysis and death were noted. Relaxant activity was performed on isolated rabbits’ jejunum preparations mounted in 10 ml tissue bath under controlled temperature 37 ± 1 °C constantly aerated with cabogen gas. The extract was tested at concentrations of 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 5.0 and 10.0 mg/ml on spontaneous rabbits’ jejunum preparations. In similar concentrations, Vt. Cr was also tested on KCl (80 mM) induced contractions. Relaxant activity was quantified against atropine, a standard relaxant. While attempting for possible mode of action as relaxant, calcium chloride curves were constructed in K-rich Tyrode’s solution. The effects of Vt.Cr were tested on calcium chloride curves at concentrations 3.0 mg/ml and 5.0.0 mg/ml. Curves for Verapamil at concentrations 0.1 and 0.3 µM were also constructed. The curves were compared with their respective control for possible right shift.
Results

Preliminary phytochemical tests resulted in the presence of flavonoids, saponins, tannins, terpenoids, glycosides, carbohydrates, proteins, fats and fixed oils.

Time taken for paralysis of Ascaridia galli was shorter for higher concentrations of Vt.Cr, i.e., 25 ± 2.3 minutes at 40 mg/ml. Whereas, time taken for paralysis of Raillietina spiralis was 28 ± 3 minutes at 40 mg/ml of test samples of Vt.Cr. The time taken for paralysis was compared with time taken by standard albendazole (10 mg/ml). Relative index values for Ascaridia galli were 4.58, 3.41 and 2.08, for respective concentrations, i.e., 10, 20 and 40 mg/ml. Whereas, relative index for death of Ascaridia galli was 1.09 at concentration 40 mg/ml, suggesting that Vt.Cr is wormicidal at high concentration. Similarly, relative indexes for paralysis and death against Raillietina spiralis were, respectively, 1.33 and 0.824 at concentration 40 mg/ml, suggesting that Vt.Cr was more potent wormicidal than albendazole at concentration 40 mg/ml. In case of relaxant activity, mean EC\textsubscript{50} values for spontaneous and KCl induced contractions were 7.5 ± 1.4 mg/ml (6.57-8.01, n=6) and 7.9 ± 0.41 mg/ml (7.44-8.46, n=6), respectively. The relaxant activity of the Vt.Cr was 11.42 ± 2, 17.0 ± 3, 28.5 ± 4, and 128.0 ± 7 % of atropine maximum at corresponding concentrations, i.e., 1.0, 3.0, 5.0 and 10 mg/ml. While constructing calcium chloride curves, Vt.Cr at 3 mg/ml, had mean EC\textsubscript{50} \((\log \text{ calcium molar}) = -1.9 ± 0.06 (-1.87 – -1.98, n=6)\) vs. control EC\textsubscript{50} = -2.5 ± 0.12 (-2.37 – -2.56, n=6). Results for 0.1 µM verapamil were EC\textsubscript{50} = -1.7 ± 0.1 (-1.6 – -1.8, n=6) vs. control EC\textsubscript{50} = -2.4 ± 0.09 (-2.3 – -2.47, n=5).

Conclusions

Anthelmintic activity of Verbascum thapsus confirms its ethnobotanical use in some local tribe of the Malakand region. Moreover, the relaxant effect of Verbascum thapsus also justifies its traditional use in management of abdominal pain as antispasmodic.
Background

The family, “Scrophulariaceae”, commonly known as Figwort Family, consists of 269 genera and 5100 species, located in temperate and tropical mountains [1]. The plants of the family are herbs or sub shrubs. The genus Verbascum belongs to Figwort Family and is represented by 360 species in the world [2]. Plants of this genus are widely used in folk medicine [3], and are, therefore, of great pharmacological importance. The leaves and flowers of Verbascum (scrophulariaceae) are reported to have expectorant, mucolytic and demulcent properties, which are used to treat respiratory disorders like bronchitis, dry coughs, tuberculosis and asthma in traditional Turkish medicine. Plants belonging to genus Verbascum are also used to treat hemorrhoids, rheumatic pain, superficial fungal infections, wounds and diarrhea. They have inhibitory activities against the murine lymphocytic leukemia and influenza viruses A2 and B [4]. Likewise, Verbascum phlomoides contains iridoid ester glycoside, known as specioside. It also contains caffeic acid esters, verbascoside and forsythoside B; saponins that are known as desrhamnosylverbascosaponin [5], verbascoside [3]. Verbascum phlomoides and Verbascum densiflorum are reported to contain iridoids as aucubin, catalpol, 6-O-β-D-xylopyranosylaucubin and saccatoside. In addition to the leading iridoids aucubin and catalpol, Verbascum densiflorum also contains harpagide, harpagide acetate, and 6-O-(4″-p-methoxy-trans-cinnamoyl)-α-L-rhamnopyranosylcatalpol [6]. Extracts of Verbascum gypsicola and Verbascum sinuatum have shown significant antimicrobial activity [7, 4].

Traditionally, the leaves, flowers and roots of Verbascum species are used for the treatment of fever and bleeding of lungs. They are also used as astringent, and narcotic [8]. The overall plant is used in the treatment of diarrhea and dysentery. It is also used as
analgesic, antiseptic and wound healer. The paste of leaves and flowers are used against cough and pulmonary diseases while its seeds are narcotic [9-11]. Leaves’ powder in the form of poultice is used to relieve joints’ pain and for softening of boils [12]. Dried leaves of *Verbascum thapsus* are smoked for mental relaxation while the tea of its leaves is used in cold and dysentery. The smoke of the plant is utilized to drive away the ghostly instincts from the children [13]. Decoction of its dried leaves and flowers are used in the treatment of sore throat, bronchitis, expectorant, sedative and abdominal pain [14]. The leaves and flowers are used as expectorant in bronchitis, antispasmodic, tuberculosis and other respiratory ailments. Leaves are smoked to ease chest complaints and asthma [15].

**The present research paper** describes the scientific rationale for use of *Verbascum thapsus* in abdominal pains as relaxant (antispasmodic activity), and anthelmintic activity.

**Methods**

**Collection, Identification and Preparation of Plant Materials**

Fresh aerial parts of *Verbascum thapsus* (300g) were collected from nearby hills of Chakdara campus of University of Malakand. Professor Dr. Jehandar Shah, Vice Chancellor, Shaheed Benazir Bhutto University, Sheringal Dir Upper, located and identified the plant. A voucher specimen Vt-01-2009 has been submitted to the herbarium of, University of Malakand. The fresh aerial parts were gently washed with distilled water. The materials were subjected to shade drying. After shade drying, the materials were crushed and macerated in 1.0 liter commercial grade methanol for 3-4 days. The materials were then filtered through ordinary filter paper. The process was repeated thrice. The filtrates were combined and evaporated under reduced pressure using a rotary evaporator at 40 °C till a solvent free semisolid extract was obtained (yield = 9.9%).
Drugs and Animals

Anthelmintic activity was determined using adult round worms (*Ascaridia galli*) and tape worms (*Raillietina spiralis*), identified by veterinary practitioner and zoologist of Biotechnology Department of University of Malakand. Fresh infested intestines of the fowls were collected from the nearby slaughter house of Chakdara. The tissues were kept in normal saline. Tape worms and round worms were isolated from the intestines. The parasites were maintained in normal saline for the experimentations. The average size of earthworm was in range of 6-8 cm, average size of tape worm was in range of 6 - 7.8 cm and the average size of round worm was in range of 4.8- 7 cm. Albendazole (Glaxo Smith Kline) was used as standard anthelmintic drug [16].

Unless specified, analytical grade chemicals were used in the experiments purchased from E. Merck. Acetylcholine was purchased from BDH Chemicals, Poole, England. All solutions were prepared in distilled water on corresponding days of experiments. The rabbits were purchased from local market and bred at the animal house of University of Malakand. They were treated as per the principles mentioned in the “Animals Byelaws 2008 of the, University of Malakand (Scientific Procedures Issue-I)”. Ethical Committee of the department of pharmacy, constituted under the approved Animals Byelaws 2008 of the, University of Malakand, endorsed the study protocols.

Preliminary Phytochemical Screening

Preliminary phytochemical tests for the powdered materials and extract of *Verbascum thapsus* were carried out for the presence of flavonoids, saponins, tannins, glycosides,
cardiac glycosides, carbohydrates, proteins, and sterols according to the standard procedures [17-19].

**Anthelmintic Activity**

Ajaiyeoba, et al’s method was used to determine the anthelmintic activity of *Verbascum thapsus* [20]. Fresh adult round worms (*Ascaridia galli*) and tape worms (*Raillietina spiralis*) were used in experimentations for evaluation of anthelmintic activity [21-23]. Test samples of the extract were prepared at concentrations of 10, 20 and 40 mg/ml in normal saline. Six test worms of both the species, approximately of equal size were placed in petri dish containing 25 ml of the above test solutions of Vt.Cr. Albendazole (10 mg/ml) and distilled water having six test worms, each, were used as positive standard and negative control, respectively. All test solutions and standard solutions were freshly prepared at the time of corresponding experiment. Observations were made for the time taken for paralysis. Paralysis was declared from time when every sort of movement was stopped except when the worms were shaken, vigorously. However, time for death of the worms was recorded when the worms neither moved upon vigorous shaking nor upon dipping in warm water (50 °C). The experiments were carried out in quadruplicate and observations were recorded.

**Recording of Electrophysiological / Electro Pharmacological Effects**

Force transducer (MLT 0210/A) connected with Power Lab ADInstruments Australia was used to record the tissues response. Other setting parameters were 5Hz × 10 gain (input 1) @40/S, low pass, range 20 mv.
Effects on Spontaneous Rabbits’ Jejunum Preparations

Rabbits of either sex (average weight = 1.8 ± 0.2 kg) were purchased from local market. They were bred at the animal house of the University of Malakand. They were kept starved for 24 hours prior to start of the experiments. They had a free access to water. Their abdomens were opened after cervical dislocation. Pieces of jejunums were removed and maintained in a petri dish constantly aerated with carbogen (95% oxygen: 5% Carbon dioxide) gas [24, 25]. Rabbits’ jejunum preparations (1-1.5 cm) were mounted in 10 ml tissue bath containing normal Tyrode’s solution. The composition of normal Tyrode’s solution (mM) was KCl 2.68, NaCl 136.9, MgCl\(_2\) 1.05, NaHCO\(_3\) 11.90, NaH\(_2\)PO\(_4\) 0.42, CaCl\(_2\) 1.8 and glucose 5.55. The tissues were maintained at 37 ± 1 degree Celsius, constantly bubbled with carbogen gas. The tissues were stabilized for 20 minutes. After stabilization when it gave reproducible responses, the Vt.Cr was tried at concentrations 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 5.0 and 10.0 mg/ml [24, 25]. Relaxant response of Vt.Cr was quantified against standard atropine at concentration 1.0 µM. Responses were recorded.

Effects on KCl (80 mM)-Induced Contractions

While attempting to explain its possible mode of action, the Vt.Cr was also tried in similar concentrations, i.e., 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 5.0 and 10.0 mg/ml on sustained contractions induced by KCl (80 mM) [24, 25]. Earlier, the tissues were stabilized in normal Tyrode’s solution for at least 30 minutes. Responses were noted.

Effects of Extract on Calcium Chloride Curves

To confirm the possible mode of action for relaxant activity, control calcium chloride curves were constructed in decalcified tissues. In brief, the tissues were stabilized in 10
ml tissue bath containing Tyrode’s normal solution. The tissues were then exposed to K-normal Tyrode’s solution followed by K-rich Tyrode’s solution [24, 25, 26]. Constituents and concentration (mM) of K Rich Tyrode’s solution was KCl 50, NaCl 91.04, MgCl₂ 1.05, NaHCO₃ 11.90, NaH₂PO₄ 0.42, glucose 5.55 and EDTA 0.1. This led to decalcification of the tissues, which were constantly bubbled with carbogen gas at 37 ±1°C. The tissues were calcified with known concentrations of calcium in a range of 1×10⁻⁴ – 256 ×10⁻⁴ Molar concentrations of calcium. Thus standard control curves were constructed. The tissues were treated with known concentration of Vt.Cr (3.0 mg/ml, and 5.0 mg/ml). Following an incubation period of one hour, the researchers constructed calcium chloride curves in similar manner. Likewise, control curves for verapamil (0.1 and 0.3 µM) were constructed. EC₅₀ values of crude methanol extract of Verbascum thapsus were compared with respective controls for possible right shift.

**Statistical Interpretation and Reading of Chart Data**

Chart 5 was used to interpret the electrophysiological data purchased from ADInstruments, Australia. “Student’s t-test” was used to compare the significance at 95% confidence interval. P value less or equal to 0.05 was considered statistically significant. Graph Pad Prism and XL sheet was used to draw the curves. EC₅₀ values and standard error of mean (SEM) were calculated at 95 % confidence interval.

**Results and Discussion**

Phytochemical tests revealed the presence of flavonoids, saponins, tannins, terpenoids, glycosides, carbohydrates, proteins, fats and fixed oils.

Results of anthelmintic activity are summarized in Table 1. It is clear that at higher concentration, Vt.Cr produced paralysis of the test worms. Time taken for paralysis of
*Ascaridia galli* is shorter for higher concentrations of Vt.Cr i.e. 25 ± 2.3 minutes at 40 mg/ml. While time taken for paralysis of *Raillietina spiralis* was 28 ± 23 minutes at 40 mg/ml of test samples of Vt.Cr. Relative index values for *Ascaridia galli* are: 4.58, 3.41 and 2.08, for respective concentrations, i.e., 10, 20 and 40 mg/ml vs. standard albendazole (10 mg/ml). Whereas, relative index for death of *Ascaridia galli* was 1.09 at concentration 40 mg/ml, suggesting that anthelmentic activity of Vt.Cr is comparable with standard albendazole at high concentration (40 mg/ml). Similarly, relative index for paralysis and death against *Raillietina spiralis* are, respectively, 1.33 and 0.824 at concentration 40 mg/ml. This demonstrates that the Vt.Cr is even more potent wormicidal than albendazole against *Raillietina spiralis*. The experimental evidence, mentioned in Table 1, can be used for activity guided isolation of bioactive compounds from the *Verbascum thapsus*.

While testing on spontaneous rabbits’ jejunum preparations, the Vt.Cr produced concentration dependent relaxation of the spontaneous contractions (Figure 1). Mean EC50 values for spontaneous and KCl induced contractions are 7.5 ± 1.4 mg/ml (6.57-8.01, n=6, P < 0.001 vs. control), and 7.9 ± 0.41 mg/ml (7.44-8.46, n=6), respectively. The relaxing effects of Vt.Cr were quantified against atropine. The relaxant activity of the Vt.Cr was 11.42 ± 2, 17.0 ± 3, 28.5 ± 4, and 128.0 ± 7 % (P < 0.001) of atropine maximum at its corresponding concentrations, i.e., 1.0, 3.0, 5.0 and 10 mg/ml (Figure 2). Intracellular and extracellular stores of calcium exchange with each other through voltage operated calcium channels that help in regulating spontaneous intestinal responses. It is noteworthy that the contractile effects of the intestine are due to free cytosolic calcium levels that enter into sarcoplasmic reticulum through voltage operated calcium channels.
[26]. Thus blocking of exchange of calcium through voltage operated channels will relax the tissues. The results suggest that the mode of action is possibly through calcium channels, as high molar KCl induced contractions are usually, not necessarily, through calcium channels [25-27]. While confirming the mode of action, the researchers took the support of constructing calcium chloride curves (Figure 3). Vt.Cr at concentration 3 mg/ml shifted the calcium curves to right, i.e., Vt.Cr at 3mg/ml, had mean EC$_{50}$ = -1.9 ± 0.06 (-1.87 – -1.98, n=6) vs. control EC$_{50}$ = -2.5 ± 0.12 (-2.37 – -2.56, n=6) log calcium molar (P < 0.0001, i.e., extremely significant). The right shift showed that some of the calcium channels were blocked and calcium could not enter the cell through voltage sensitive L type calcium channels [24,25,26,27]. Similarly, construction of calcium curves in presence of verapamil 0.1 µM gave EC$_{50}$ (log calcium molar) = -1.7 ± 0.1 (-1.6 – -1.8, n=6, P =0.0004, i.e., extremely significant) vs. control EC$_{50}$ = -2.4 ± 0.09 (-2.3 – -2.47, n=5). The right shift of test samples of Vt.Cr resembles the right shift of verapamil curves. Therefore, it is confirmed that mode of relaxation is through voltage sensitive L-type calcium channels of the tissues. The plant species could be a potential target for isolation of bioactive constituent(s).

**Conclusions**

The present research work confirms the relaxant effect of *Verbascum thapsus* that justifies its use in management of abdominal pain as spasmolytic. The plant is also effective as anthelmintic that confirms its ethnobotanical use in some local tribes of Malakand region.
Acknowledgment

The authors pay rich thanks to Professor Dr. Jehandar Shah for locating and identifying the Plant. The authors also want to thank Mr. Tariq Khan Sr. faculty member, Department of English, University of Malakand, for language corrections of the manuscript.

Competing Interests

The author(s) declare that they have no competing interests.

Authors’ Contributions

NA participated in data collection of relaxant activity, data interpretation and preparation of the manuscript. SWA helped in data collection of anthelmintic activity and in writing the introduction section of the manuscript. IS also helped in data collection. Similarly, GA also helped out in data collection. MG was out there in data collection and maintenance of laboratory animals. Last but not the least IK also participated actively in data collection.

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Legends

Figure 1: Effects of Vt.Cr on spontaneous and KCl-induced contractions (All Values are mean ± SEM, n=6, *P* < 0.05 vs. control maximum).

Figure 2: % Relaxation of atropine maximum in rabbits’ jejunum preparations (All values are mean ± SD, n=5, *P* < 0.05, **P** < 0.01 and ***P** < 0.001 vs. atropine maximum).

Figure 3: (A) Calcium chloride curves in the absence and presence of Vt.Cr

(B) Calcium chloride curves in the absence and presence of verapamil.

(All values are mean ± SEM, n=6, *P* < 0.05)
Table 1: Anthelmintic activity of extract of *Verbascum thapsus* against *Ascaridia galli* and *Raillietina spiralis*.

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<tr>
<th>S. No</th>
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<th>Concentrations (mg/ml of normal saline)</th>
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<td></td>
<td><em>Ascaridia galli</em></td>
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<td><em>Raillietina spiralis</em></td>
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<td>15 ± 1.8</td>
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<td>1.09</td>
<td>21 ± 2.5</td>
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Key:

Relative index (P) = Time taken by samples for paralysis / Time taken by standard *for paralysis. Relative index (D) = Time taken by samples for death / time taken by standard *for death. Time was recorded in minutes.
Figure 1

% of Control Max.

- Spontaneous
- KCl (80 mM)

Vt.Cr (mg/ml)
Figure 3

Graph A and B show the relationship between the logarithm of calcium concentration ([Ca^{++}]M) and the percentage of control max. The graphs illustrate the effects of different concentrations of a substance (Control, 3.0 mg/ml, 5.0 mg/ml) and a drug (0.1 Micro Molar Verapamil, 0.3 Micro Molar Verapamil) on the percentage of control max. The graphs indicate statistically significant differences marked by an asterisk (*).
Additional files provided with this submission:

Additional file 1: Dr. Niaz Ali's Manuscript. R4- track changes on.doc, 120K
http://www.biomedcentral.com/imedia/1619873406660177/supp1.doc