

Receptor based study of ethanolic seed extract of *Butea frondosa* Roxb. Ex. Willd for involvement of cholinergic pathway in regulation with NO signaling to establish a potential anti-trematodal drug against *Paramphistomum cervi*

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Abstract

Purpose

Reports were found for traditional use of *Butea frondosa* Roxb. Ex. Willd for nematode infection but its anti-trematodal property and mechanistic action as an anthelmintic is not yet established. Therefore, present study was conducted to evaluate the same as an anti-trematodal agent.

Methods

Mature and healthy *Paramphistomum cervi* were collected from the rumen of freshly slaughtered cattle of local abattoir in warm HBSS. For *in vitro* motility study, six adult *P. cervi* in each Petri dish having different dilutions of extracts in HBSS ranging from 50, 150, 300, 500, 1000 and 3000 µg/ml and standard Oxytocin (10⁻⁵ M) with control receiving only HBSS were taken and incubated at 38 ± 1° C for 5 h. Based on their gross visual motility, ethanolic seed extract of *B. frondosa* Roxb. Ex. Willd in various concentrations (100, 300, 1000 and 3000 µg/ml) were added to the isometrically mounted *P. cervi* on isolated tissue bath and their effects on spontaneous muscular activity of *P. cervi* were estimated.

Results

Ethanolic seed extract of *B. frondosa* Roxb. Ex. Willd @ 1000 and 3000 µg/ml showed complete death of worms within 1 hr of exposure which was comparable with the standard drug Oxytocin @10⁻⁵ M. For neuropharmacological evaluation on isolated tissue bath, ethanolic seed extract of *B. frondosa* Roxb. Ex. Willd was found to be hyperpolarizing in nature and caused paralysis and death of worms @ 3000 µg/ml.

Introduction

The typical pharmacological basis for treating helminths typically entails interfering with one or more of the parasite's essential functions, such as impairing the process by which energy is generated, rupturing the neuromuscular coordination that renders the parasites paralyzed, and having a negative impact on the reproductive process.

Neurotransmitters are endogenous chemicals found in helminth parasites and mammals that are produced from nerve terminals at synapses and neuromuscular junctions in a selective manner [1]. The neurology of parasitic helminths is a field with very little knowledge. Pharmacological investigation is the only way to understand their differences from vertebrate receptor. It has not been possible to sufficiently characterize any ion channel or second messenger in parasitic helminths. The biochemical reason for the distinction between the contractile function of helminth muscle and vertebrate smooth and skeletal muscle is yet unknown. Little is known about the fundamental biology of neuromuscular function in

helminthes, despite the fact that the neuromuscular system of parasitic helminths is analogous to human pharmacology and may therefore present a potentially appealing target for chemotherapeutic intervention. As a result, the process of finding anthelmintic remains challenging and inefficient. Commercial anthelmintics are the mainstay of the control of gastrointestinal helminth infection in a country like India. Nonetheless, a number of factors, most notably anthelmintic resistance and the existence of medication residues in milk, meat, wool, and most importantly pasture, render cattle particularly susceptible to long-term and cumulative toxicity. The best moment to create a complementary herbal remedy to effectively lessen such circumstances has arrived now. Humans rely entirely on nature to maintain ecological balance, but nature has also given us a priceless gift in the shape of medicinal plants that are used by many Indian tribes. Northeast India is one of the "biodiversity areas of interest," forming a new biogeographic domain that holds onto important biomes recognized on the world, and it has the most abundant supply of plant variety in all of India.

Palash (*Butea frondosa* Roxb. Ex. Willd) is medium-sized deciduous tree, 10–15 meters high belonging to the family Fabaceae, is found through- out India [2]. There are four types of Palash viz. Rakta (red), Pita (yellow), Shweta (white) and Nila (blue) as mentioned by Narahari in Raj Nighantu [3]. Chemical component of *B. frondosa* are alkaloids and recently reported Euphane triterpenoid ester and pterocarpan. Seed contains palasonin, d-methyl cantharidin, α -amyrin, β -sitosterol and alkaloid-monospermine. Glycerides of palmitic, stearic, linoceric, oleic and linoleic acids, proteolytic and lipolytic enzymes. *B. frondosa* popularly referred to as 'flame of the forest', has exhibited excellent anthelmintic property especially for roundworms [4]. [5] reported anthelmintic activity of *B. frondosa* (Koeing ex Roxb.) seeds extracts against Benzimidazole resistant caprine gastrointestinal nematodes. [6] reported anthelmintic activity, toxicity and other pharmacological properties of palasonin, the active principle of *B. frondosa* seeds and its piperazine salt. Kymographic studies with normally expelled human *Ascaris* indicated that palasonin was more effective against *Ascaris lumbricoides* than piperazine or santonin, whilst the piperazine salt of palasonin was much more active than either palasonin or piperazine alone.

Materials and methods

Ethics statement:

The *in vivo* assays were conducted in accordance with the internationally accepted principles for laboratory animal use and care of laboratory animals by the National Academic Sciences. The study was conducted after approval from the Institutional Animal Ethics Committee (IAEC), AAU, Khanapara, vide approval No. 770/GO/Re/S/03/CPCSEA/ FVSc/AAU/IAEC/21–22/941 dated 20.08.2022.

Collection of seeds of *Butea frondosa* Roxb. Ex. Willd and authentication:

Butea frondosa Roxb. Ex. Willd seeds were obtained from Arunachal Pradesh and rural Assam. The seed material was authenticated by Dr. I. C. Barua, Principal Scientist from the Department of Agronomy, Assam Agricultural University, and the voucher specimen (6571, dated 14 September 2021) was

preserved in their institutional herbarium and deposited in the Botanical Survey of India herbarium. The samples were identified by Botanical Survey of India, Meghalaya, Shillong.

Preparation of ethanolic seed extract of *B. frondosa* Roxb. Ex. Willd (ESEBF):

In a glass beaker, precisely 250 g of powdered seed material was soaked in 1000 ml of ethanol for 72 h. Using a sterile glass rod, the liquid was swirled every 24 h. After going through muslin fabric, the filtrate was obtained and concentrated at 45–50° C under decreased pressure in a rotary evaporator (EQUITRON, Roteva, made by MEDICA INSTRUMENT MFG. CO. Mumbai–400013). To achieve a semi-solid consistency, the extract was further dried over a water bath at 37° C. Until it was used, the extract was stored at 4° C in an airtight container with suitable labelling.

Collection of *Paramphistomum cervi*:

Mature and healthy *Paramphistomum cervi* were collected from the rumen of freshly slaughtered cattle at local abattoir in warm (37 ± 1° C) Hanks Balanced Salt Solution (HBSS) in an insulated container and brought to the laboratory. The worms were kept in the BOD incubator at 37 ± 1° C until further use. The flukes were identified before experimentation using the procedure followed by [7].

Acute toxicity study:

An acute oral toxicity study was performed according to the guidelines of OECD-423. The overnight fasted mice (n = 3) were orally administered ESEBF at the limit dose of 2000 mg/kg body weight and observed continuously for behavioral, neurological and autonomic profiles for 30 minutes, 1h, 2h, 4h, 6h and then 24h and 72h and thereafter up to 14 days for any lethality, moribund state or death.

In vitro motility study:

Six adult *P. cervi* in each Petri dish having different dilutions of extract (ESEBF) in HBSS ranging from 50, 150, 300, 500, 1000 and 3000 µg/ml and standard Oxyclozanide (10^{-5} M) were taken. However, control Petri dish received only HBSS. The total volume of HBSS in each Petri dish was kept at 5 ml. It was then incubated at 37 ± 1° C for 5 h and the number of live and dead adult worms were counted at 0, 30, 60, 90, 120, 150, 180, 210, 240, 270 and 300 minutes of exposure as described by [8].

% corrected mortality = Total mortality – mortality in the control group/ Total mortality

Experimental procedures and mounting of *P. cervi* on isolated tissue bath:

At 37 ± 1°C, the adult *P. cervi* was positioned isometrically in HBSS solution [9]. Two little hooks were used to help mount the worm. A tension of 200 mg was applied to the parasite using two hooks: one was inserted 12 mm caudal to the anterior sucker and fixed to the tip of the aeration tube; the other was pierced through the surface of the acetabulum and connected to the isometric force transducer (Powerlab, 4/35, 4Channel Data Acquisition System, Model: ML866/P, AD Instruments, Australia). Following setup, the samples were left to acclimatise in bath fluid for forty-five minutes, with a standard

20-minute interval between drug exposures. Following equilibration, the tissue bath was supplemented with ESEBF at different doses (100, 300, 1000, and 3000 µg/ml) in order to observe how they affected spontaneous muscle activity of *P. cervi*. Three parameters—amplitude, baseline tension, and frequency—were measured for ten minutes and recorded in the Chart Window 7 software programme. Before adding different concentrations of ESEBF, control recordings were made for 15 minutes.

Evaluation of effect of ESEBF with the excitatory neurotransmitters and/ or antagonist of inhibitory neurotransmitters on the SMA of *P. cervi*:

ESEBF @ 3000 µg/ml was combined with different excitatory neurotransmitters as well as antagonists of inhibitory neurotransmitters, in the tissue bath containing *Paramphistomum* to establish the interaction between the agents and finally their effects on SMA of *P. cervi* were estimated.

Statistical analysis:

The results are presented as mean ± standard error. Level of significance was analysed by using SPSS version 20.0 using one- and two-way ANOVA.

Result and discussion

Percentage yield of extract obtained from seeds of *B. frondosa* Roxb. Ex. Willd:

The percentage yield of the solid residue obtained during the extraction of ESEBF was found to be 14.35% w/w respectively

Acute toxicity study:

The study revealed no lethality or toxic manifestation after oral administration of ESEBF up to the limit dose *i.e* 2000 mg/kg body weight in mice. Similar finding was reported by [10] with no lethality or toxicity profile after oral administration of limit dose of hydroethanolic extract of *B. frondosa* in albino rats.

Gross visual motility of *P. cervi*:

Reduction of motility of parasite was evident as feeble at 5 h of exposure in presence of Oxytoclozanide (10^{-5} M). Similar type of result has also been observed with the ESEBF @ dose rate of 1000 and 3000 µg/ml. The results are presented in Table: 1.

In gross motility study, ESEBF @ 1000 and 3000 µg/ml was as effective as Oxytoclozanide @ 10^{-5} M. Similar findings was reported in *G. crumenifer* with the alcoholic extract of *M. philippinensis* (300 and 1000 µg/ml), *B. frondosa* (1000 and 3000 µg/ml), *N. sativa* (3000 µg/ml), essential oil of *E. grandis* (1000 µg/ml), *N. sativa* (300 µg/ml), *C. deodar* (1000 µg/ml) [11].

Effect of cumulative addition of ESEBF on spontaneous muscular activity (SMA) of *P. cervi*:

In isometrically mounted mature *P. cervi*, the control (prior to application of drug) amplitude, baseline tension and frequency of spontaneous muscular contractions were recorded to be 0.42 ± 0.06 g, 0.20 ± 0.03 g and 53.72 ± 5.49 per 5 min respectively. The ESEBF produced hyperpolarizing effect on rhythmic contraction of *P. cervi*. The amplitude of the *P. cervi* reduced significantly at 300 μ g/ml ($P < 0.05$) and at 1000 to 3000 μ g/ml ($P < 0.01$) concentrations. The baseline tension of the SMA was reduced significantly at 1000 μ g/ml ($P < 0.05$) and at 3000 μ g/ml ($P < 0.01$) concentrations as compared to control value. Likewise, frequency of the SMA was reduced significantly at 300 μ g/ml ($P < 0.05$) and at 1000 to 3000 μ g/ml ($P < 0.01$) concentrations as compared to control. The representative recordings are given in Table 2 and Figs. 1, 2, 3, 4 and 5.

Table 1
In vitro gross motility of ESEBF against *P. cervi*

Drug/Extract	Conc (μ g/ml)	No of parasite showing motility (in minutes)										
		0	30	60	90	120	150	180	210	240	270	300
Control (HBSS)	-	6	6	6	6	6	6	6	6	6	6	6
B. frondosa Roxb. Ex. Willd (Ethanolic Extract)	50	6	6	6	6	6	6	5	5	4	3	2
	150	6	6	6	6	5	4	3	1	1	0	0
	300	6	6	5	4	2	1	0	0	0	0	0
	500	6	5	3	1	0	0	0	0	0	0	0
	1000	6	4	2	0	0	0	0	0	0	0	0
	3000	6	3	1	0	0	0	0	0	0	0	0
Oxyclozanide	10^{-5} M	6	2	0	0	0	0	0	0	0	0	0

Table 2
Effect of ESEBF on amplitude (g), baseline tension (g) and frequency (per 5 min) of SMA of *P. cervi*

Observations	Concentrations				
	Control	100 μ g/ml	300 μ g/ml	1000 μ g/ml	3000 μ g/ml
Amplitude (g)	0.42 ± 0.06	0.37 ± 0.06	$0.32 \pm 0.05^*$	$0.26 \pm 0.04^{**}$	$0.20 \pm 0.04^{**}$
Baseline tension (g)	0.20 ± 0.03	0.18 ± 0.02	0.15 ± 0.02	$0.14 \pm 0.03^*$	$0.11 \pm 0.03^{**}$
Frequency/5min	53.72 ± 5.49	49.99 ± 5.14	$43.65 \pm 6.55^*$	$37.06 \pm 5.56^*$	$31.55 \pm 6.05^{**}$
Values are the Mean $\pm$ S.E for six replicates.					
*P < 0.05; **P < 0.01 as compared to control.					

Many selective anthelmintic drugs target the neuromuscular system of helminth parasites in addition to their effects on the parasites' energy-generating system and reproduction [12]. Helminth parasites become paralyzed when exposed to certain drugs, such as pyrazine [13], levamisole [14], piperazine [15], avermectin [16], anthelmintic organophosphates [17], and paziquantel [18].

Due to the plant extract's quick action, alterations in the spontaneous muscle activity of isometrically mounted worms show that the neuromuscular system is involved; however, the effect on the parasites' energy metabolism and neuromuscular system is combined to generate changes in their gross visual motility. Thus, new drugs' anthelmintic activity might be assessed *in vitro* using gross visual motility and SMA.

The present study discusses the effect of various concentrations of ESEBF on the SMA of *P. cervi* *in vitro*. In the present study, ESEBF produced significant reduction of amplitude, baseline tension and frequency at 300, 1000 and 3000 µg/ml of the SMA of *P. cervi*. This observation was in agreement with alcoholic extract of *M. philippinensis* on *F. gigantica* [19] and *G. crumenifer* [11]. Again, from the previous study it was evident that the amplitude, baseline tension and frequency of the SMA of *P. cervi* were significantly reduced by methanolic, hydroethanolic and aqueous extract of *M. azedarach* Linn.

Thus, it is suggested that the ESEBF at 3000 µg/ml was so efficacious in its anti-trematodal property that the concentration caused complete paralysis of normal rhythmic muscular contraction of *P. cervi*, which could not be revived by after concurrent washings of the parasite.

Effect of ESEBF with the excitatory neurotransmitters and/ or antagonist of inhibitory neurotransmitters on the SMA of *P. cervi*:

The amplitude, baseline tension, and frequency of SMA, which demonstrate hyperpolarizing paralysis, were found to decrease concentration-dependently upon assessing the individual effect of ESEBF on isometrically mounted *P. cervi*. Additionally, in order to determine its mechanism of action as a hyperpolarizing agent, the highest concentration (3000 µg/ml) was combined with antagonists of inhibitory neurotransmitters (previously established as inhibitory neurotransmitters [20]), such as atropine, picrotoxin, propranolol, and 4-aminopyridine, as well as various excitatory neurotransmitters (already established as excitatory neurotransmitters in *P. cervi* [21; 22]). The hyperpolarizing characteristic of ESEBF prevented 5-HT, L-DOPA, propranolol, picrotoxin, calcium chloride, and 4-aminopyridine from blocking the depolarization effect these drugs displayed, and as a result, the combination had no effect on these neurotransmitters. The results are summarized in Table 3.

Table 3

Effect of ESEBF with the excitatory neurotransmitters (5-HT, L-DOPA, calcium chloride) and/ or antagonist of inhibitory neurotransmitters (4-Aminopyridine, Picrotoxin, Propranolol) on amplitude (g), baseline tension (g) and frequency of SMA of *P. cervi*

Drugs/Agents	Parameters		
	Amplitude (g)	Baseline tension (g)	Frequency/5min
Control	0.51 ± 0.07	0.19 ± 0.03	55.41 ± 5.82
5-HT (10 ⁻³ M)	0.78 ± 0.07*	0.27 ± 0.02*	74.37 ± 5.28*
5-HT (10 ⁻³ M) + <i>B. frondosa</i> Roxb. Ex. Willd (3000 µg/ml)	0.79 ± 0.06 ^{NS}	0.28 ± 0.02 ^{NS}	75 ± 5.06 ^{NS}
Control	0.47 ± 0.05	0.22 ± 0.04	58.89 ± 6.58
Calcium chloride (10 ⁻³ M)	0.60 ± 0.06*	0.30 ± 0.03*	68.15 ± 5.66*
Calcium chloride (10 ⁻³ M) + <i>B. frondosa</i> Roxb. Ex. Willd (3000 µg/ml)	0.61 ± 0.06 ^{NS}	0.30 ± 0.02 ^{NS}	68.94 ± 5.74 ^{NS}
Control	0.55 ± 0.05	0.21 ± 0.04	57.03 ± 5.89
L-DOPA (10 ⁻³ M)	0.79 ± 0.06**	0.32 ± 0.03**	82.52 ± 5.79**
L-DOPA (10 ⁻³ M) + <i>B. frondosa</i> Roxb. Ex. Willd (3000 µg/ml)	0.77 ± 0.05 ^{NS}	0.31 ± 0.02 ^{NS}	81.55 ± 5.15 ^{NS}
Control	0.46 ± 0.06	0.18 ± 0.04	51.28 ± 6.45
4-Aminopyridine (10 ⁻³ M)	0.68 ± 0.06**	0.29 ± 0.02**	69.41 ± 6.05*
4-Aminopyridine (10 ⁻³ M) + <i>B. frondosa</i> Roxb. Ex. Willd (3000 µg/ml)	0.69 ± 0.06 ^{NS}	0.29 ± 0.03 ^{NS}	68.47 ± 5.28 ^{NS}
Control	0.52 ± 0.07	0.22 ± 0.03	58.74 ± 6.33
Picrotoxin (10 ⁻³ M)	0.74 ± 0.06**	0.31 ± 0.02*	72.52 ± 5.77**

Values are the Mean ± S.E for six replicates.

*P < 0.05; **P < 0.01; NS (Non-significant) as compared to control.

Drugs/Agents	Parameters		
	Amplitude (g)	Baseline tension (g)	Frequency/5min
Picrotoxin (10^{-3} M) + <i>B. frondosa</i> Roxb. Ex. Willd (3000 µg/ml)	0.73 ± 0.06^{NS}	0.30 ± 0.03^{NS}	72.88 ± 6.21^{NS}
Control	0.41 ± 0.05	0.20 ± 0.04	48.72 ± 5.88
Propranolol (10^{-3} M)	$0.58 \pm 0.06^{**}$	$0.27 \pm 0.03^{*}$	$62.80 \pm 6.36^{*}$
Propranolol (10^{-3} M) + <i>B. frondosa</i> Roxb. Ex. Willd (3000 µg/ml)	0.59 ± 0.05^{NS}	0.27 ± 0.02^{NS}	61.94 ± 5.75^{NS}
Values are the Mean $\pm$ S.E for six replicates.			
*P < 0.05; **P < 0.01; NS (Non-significant) as compared to control.			

On the contrary, after addition of atropine at 10^{-3} M to the tissue bath, there was significant increase of amplitude 0.62 ± 0.06 (P < 0.01), baseline tension 0.31 ± 0.03 (P < 0.01) and frequency 72.32 ± 5.12 per 5 min (P < 0.01) respectively. The amplitude (0.28 ± 0.05 g), baseline tension (0.15 ± 0.01 g) and frequency (32.66 ± 5.42 per 5 min) were significantly (P < 0.01 and P < 0.001) reduced after addition of ESEBF at 3000 µg/ml. The representative recordings are given in Table 4 and Figs. 6 and 7.

Table 4

Combination effect of atropine (10^{-3} M) and ESEBF (3000 µg/ml) on amplitude (g), baseline tension (g) and frequency (per 5 min) of SMA of *P. cervi*

Observations	Concentrations		
	Control	Atropine (10^{-3} M)	Atropine (10^{-3} M) + <i>B. frondosa</i> Roxb. Ex. Willd (3000 µg/ml)
Amplitude (g)	0.48 ± 0.07	$0.62 \pm 0.06^{**}$	$0.28 \pm 0.05^{***}$
Baseline tension (g)	0.20 ± 0.03	$0.31 \pm 0.03^{**}$	$0.15 \pm 0.01^{**}$
Frequency/5min	51.65 ± 6.78	$72.32 \pm 5.12^{**}$	$32.66 \pm 5.42^{***}$
Values are the Mean $\pm$ S.E for six replicates.			
*P < 0.05as compared to control. **P < 0.01; ***P < 0.001as compared to atropine (10^{-3} M).			

Likewise, after addition of hemoglobin at 10^{-3} M to the tissue bath, there was significant increase of amplitude 0.65 ± 0.06 (P < 0.05), baseline tension 0.29 ± 0.03 (P < 0.05) and frequency 69.11 ± 5.79 per 5

min ($P < 0.05$) respectively. The amplitude ($0.35 \pm 0.05\text{g}$), baseline tension ($0.14 \pm 0.02\text{g}$) and frequency (34.76 ± 5.42 per 5 min) were significantly ($P < 0.01$ and $P < 0.001$) reduced after addition of ESEBF at $3000 \mu\text{g/ml}$. The representative recordings are presented in Table 5 and Figs. 8 and 9.

Table 5

Combination effect of hemoglobin (10^{-3} M) and ESEBF ($3000 \mu\text{g/ml}$) on amplitude (g), baseline tension (g) and frequency (per 5 min) of SMA of *P. cervi*

Observations	Concentrations		
	Control	Hemoglobin (10^{-3} M)	Hemoglobin (10^{-3} M) + <i>B. frondosa</i> ($3000 \mu\text{g/ml}$)
Amplitude (g)	0.52 ± 0.06	$0.65 \pm 0.06^*$	$0.35 \pm 0.05^{***}$
Baseline tension (g)	0.21 ± 0.04	$0.29 \pm 0.03^*$	$0.14 \pm 0.02^{**}$
Frequency/5min	50.12 ± 5.54	$69.11 \pm 5.79^*$	$34.76 \pm 5.42^{***}$
Values are the Mean $\pm$ S.E for six replicates.			
* $P < 0.05$ as compared to control. ** $P < 0.01$; *** $P < 0.001$ as compared to hemoglobin (10^{-3} M).			

In order to determine the likely mechanism behind the hyperpolarizing effect of ESEBF, the extract at a concentration of $3000 \mu\text{g/ml}$ was interacted with all excitatory neurotransmitters (such as 5-HT, L-DOPA, and calcium chloride, which the author had previously identified as such in *P. cervi*) and antagonists of inhibitory neurotransmitters (such as propranolol, picrotoxin, atropine, and 4-Aminopyridine, which the author had previously identified as inhibitory neurotransmitters in *P. cervi*). It was clear from the experiment that atropine was the agent that interacted with ESEBF. Atropine causes an excitatory response in *P. cervi*'s spontaneous muscle contractions by acting as an antagonist of the cholinergic receptor. Now that ESEBF ($3000 \mu\text{g/ml}$) had been added, it was clear that ESEBF had a hyperpolarizing impact on SMA of *P. cervi*. When ESEBF was added to *P. cervi* at a concentration of $3000 \mu\text{g/ml}$, it was able to significantly block its cholinergic receptor. The extract also produced such relaxation that, even after the worm was repeatedly washed with distilled water, it was completely paralyzed and died.

Furthermore, various ion channel antagonists that are particularly excitatory in nature were combined with ESEBF in order to examine the likely ion channels through which the seed extract caused the worms to become paralyzed and eventually die. ESEBF at $3000 \mu\text{g/ml}$ did not change the typical muscle contraction of CaCl_2 , indicating that it does not function through the Ca^{2+} channel when combined with CaCl_2 . Once more, the spontaneous muscle contraction of *P. cervi* was unaffected by the combination of ESEBF and 4-aminopyridine, an antagonist of the voltage-gated K^+ channel, at $3000 \mu\text{g/ml}$. This suggests that ESEBF does not also act through the K^+ channel. However, when paired with ESEBF at $3000 \mu\text{g/ml}$, hemoglobin, a NO scavenger, prevented its typical excitatory response at 10^{-3} M , and an inhibitory

response of ESEBF was visible in place of the excitatory response. Based on this discovery, it can be deduced that ESEBF at 3000 µg/ml may function via modifying the NO channel, resulting in *P. cervi* hyperpolarization and paralysis.

Discussion

The nervous system of helminths is considered to consist of the neuronal cells and nerve junctions as well as the muscles they innervate. Drugs may act at any of a number of sites important in neuromuscular systems. These includes the ionic mechanisms involved in signal transmission along nerves, the synthesis, release, and degradation of neurotransmitters and neurohormones (such as neuropeptides), the receptor on nerves and muscle that recognize these small molecules and second messenger system and ion channel that mediate the response to the arrival of information.

A neurotransmitter receptor interaction results molecular change in the receptor protein leading to either activation or inhibition of ion channels are known as ionotropic receptors. Activation or inhibition of ion channels depends on the types of neurotransmitter molecules attach to the receptor. Increasing the activity of cells (activation) if the interaction led to opening of cation channel (Na^+ and Ca^{2+} ions) whereas opening of anion channels (K^+ and Cl^-) facilitate inhibitory cellular activity.

The tetrahydropyrimidines derivatives are depolarizing neuromuscular blocking agents in trematode parasites and the vertebrate host that can on nicotinic acetylcholine receptors in nematode muscle cells. It has been reported that they produce paralysis of worms by inducing a contracture of the musculature similar to that contracture produce by acetylcholine [23]. Levamisole has a broad range of antinematode activity in numerous animal species. [24] reported that levamisole has both muscarinic and nicotinic effects. These anthelmintics act by opening of non-selective cation ion channels that are permeable to both Na^+ and K^+ and leads to increase the membrane conductance and depolarize the membrane [14].

From the perusal of literature, it was evident that no work has been undertaken to establish the involvement of cholinergic neurohumoral transmission on NO pathway modulation in the parasites. From the above findings, it was observed that ESEBF behaved similar to that of acetylcholine chloride, as it interacts with atropine and blocks the excitatory response of atropine in *P. cervi*. Again, from the different channel interaction studies, it was found that ESEBF activate NO release and cause paralysis of *P. cervi*. Now to validate these findings, [26] demonstrated the relation between acetylcholine chloride and NO release from rat spinal cord. To validate above findings, much more research is needed, so that in near future an effective anti-trematodal drug of herbal origin can be developed.

Conclusion

The results of this study may help to determine how *B. frondosa* Roxb. Ex. Willd should be used in herbal remedies to treat *P. cervi* in traditional medicine, particularly in North-East India. Additionally, by preventing the creation of resistance to synthetic anti-trematodal treatments, these findings may serve as

a guide for the development of alternative anti-trematodal medications. To confirm the reported *in vitro* anti trematodal activity, we suggest additional *in vivo* anti trematodal studies using *B. frondosa* Roxb. Ex. Willd extracts. Additionally, it is crucial to conserve *B. frondosa* Roxb. Ex. Willd for use in future experiments as well as to isolate, characterise, and test pure chemicals from the plant using TLC and HPLC for anti-trematodal action.

Declarations

Author contribution:

Conceptualization: S. Hazarika, C. C Barua, P. Mohan.

Data curation: S. Hazarika, H. Barua.

Investigation: S. Hazarika, C. C Barua, M. Hazarika.

Methodology: S. Hazarika, C. C. Barua, P. Mohan.

Formal analysis: S. Tamuli, P. Konwar.

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Writing-original draft: S. Hazarika.

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Supervision: C. C. Barua, P. Mohan.

Conflict of interest:

All authors declare no conflict of interest.

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Figures

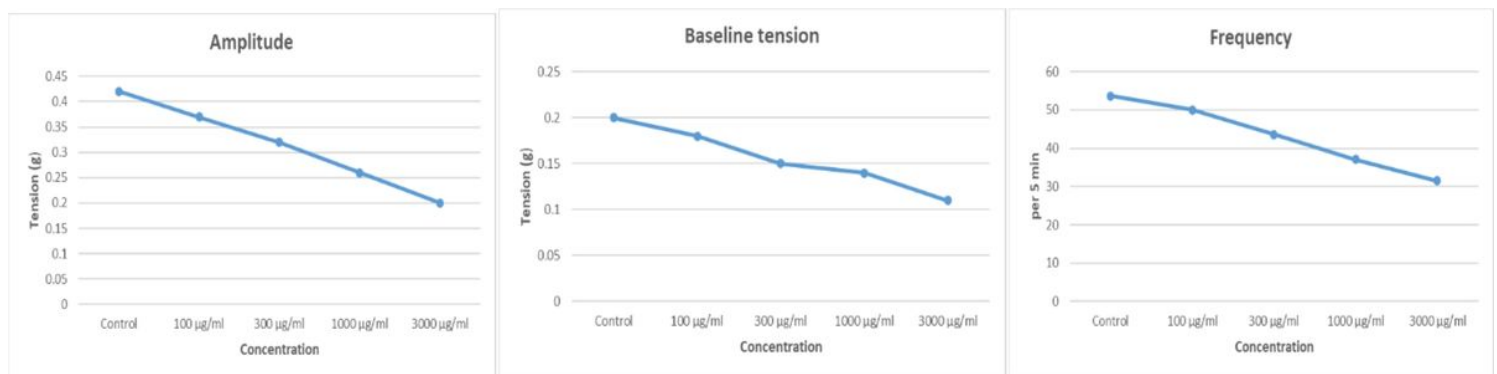


Figure 1

Graphical representation of ESEBF (100, 300, 1000 and 3000 µg/ml) on amplitude (g), baseline tension (g) and frequency (per 5 min) of SMA of *P. cervi*

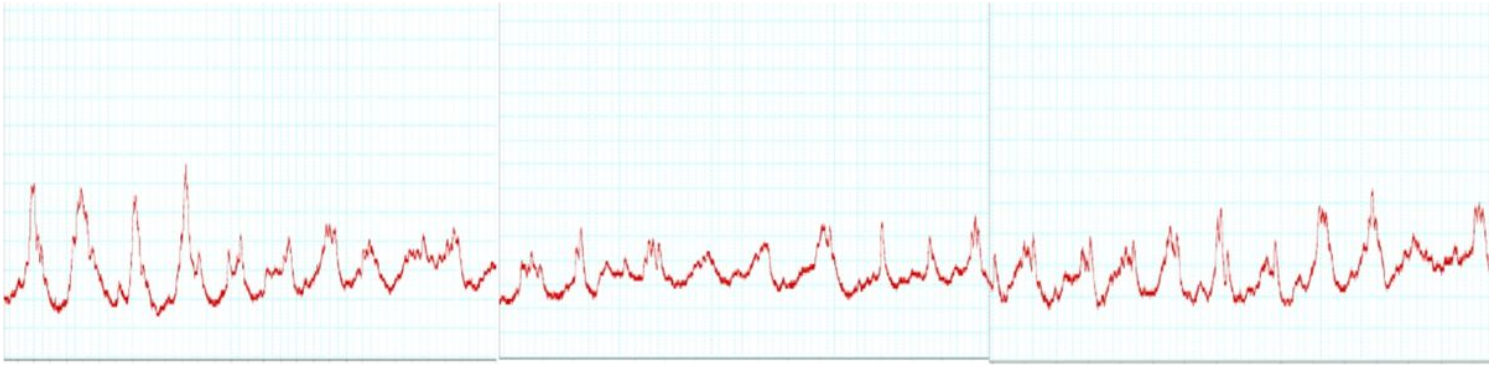


Figure 2

Effect of ESEBF (100µg/ml) on amplitude (g), baseline tension (g) and frequency (per 5 min) of SMA of *P. cervi*

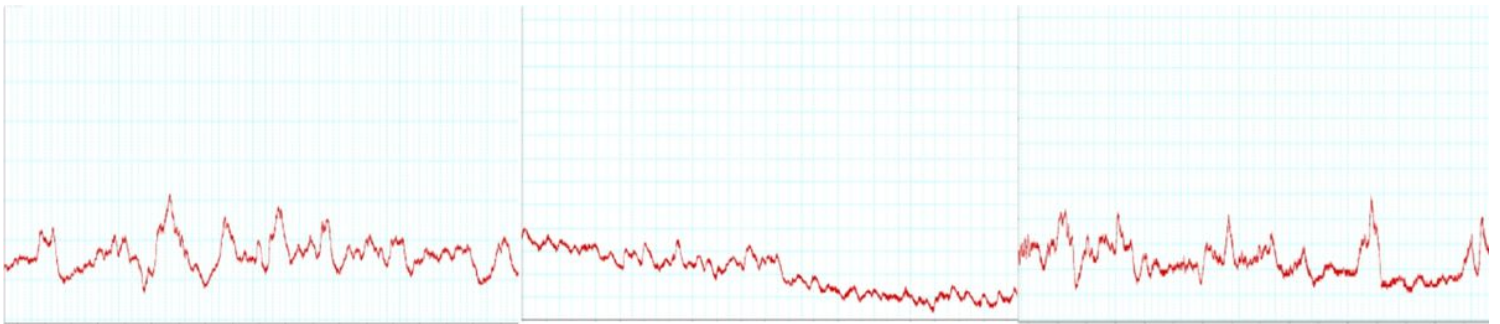


Figure 3

Effect of ESEBF (300µg/ml) on amplitude (g), baseline tension (g) and frequency (per 5 min) of SMA of *P. cervi*



Figure 4

Effect of ESEBF (1000µg/ml) on amplitude (g), baseline tension (g) and frequency (per 5 min) of SMA of *P. cervi*



Figure 5

Effect of ESEBF (3000µg/ml) on amplitude (g), baseline tension (g) and frequency (per 5 min) of SMA of *P. cervi*

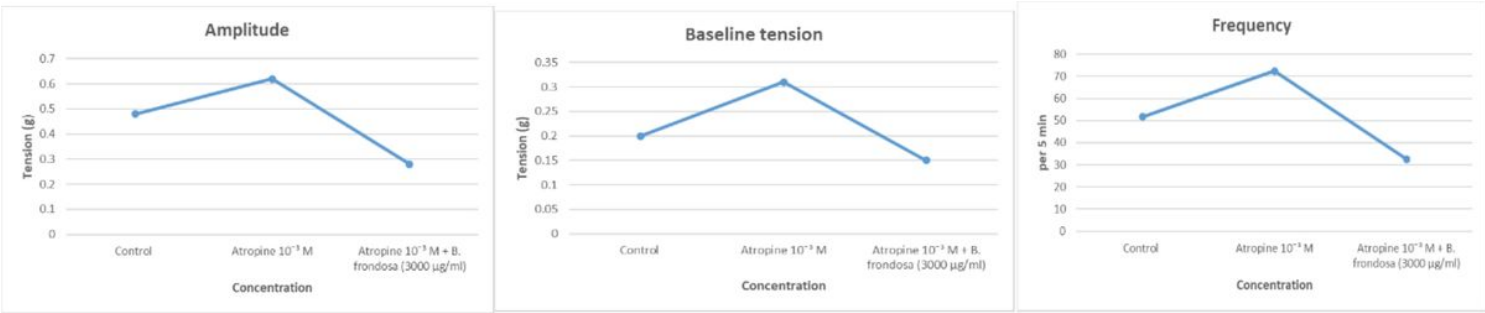


Figure 6

Graphical representation of combination effect of atropine (10⁻³ M) and ESEBF (3000 µg/ml) on SMA of *P. cervi*

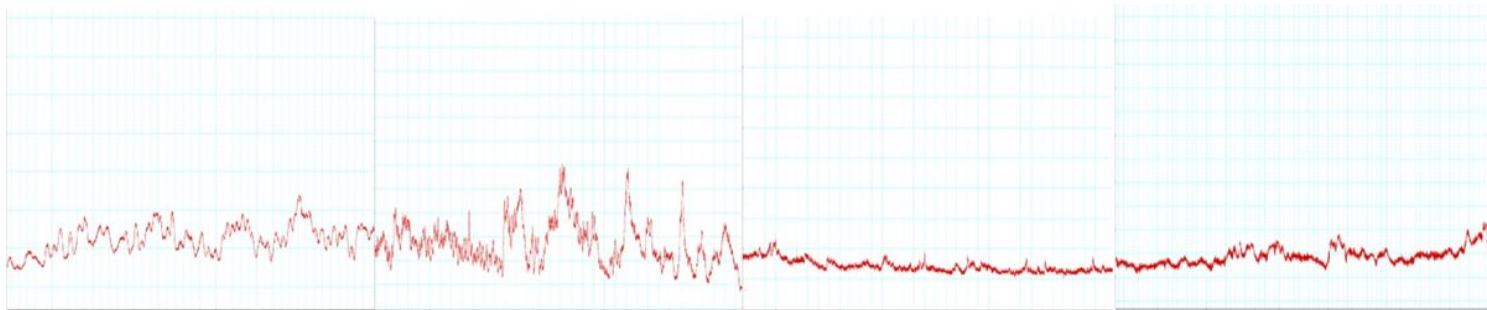


Figure 7

Combination effect of atropine (10⁻³ M) and ESEBF (3000 µg/ml) on SMA of *P. cervi*

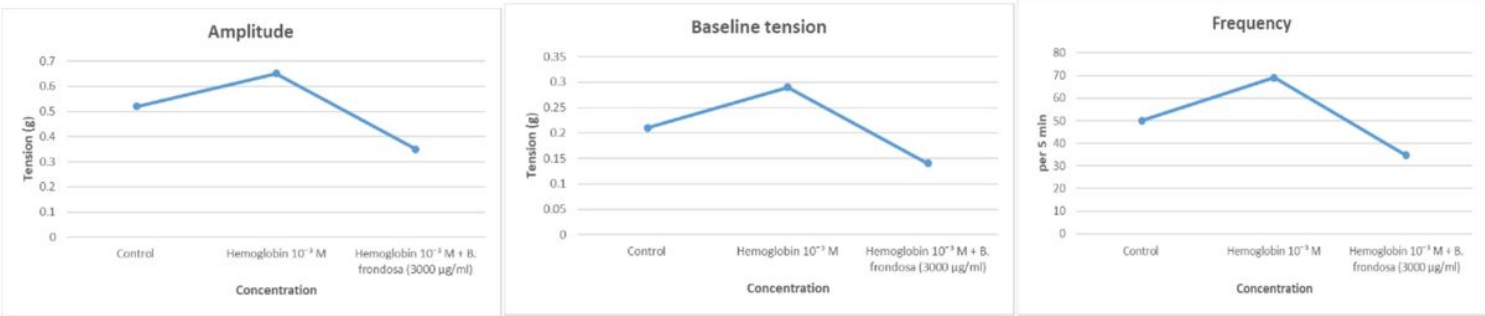


Figure 8

Graphical representation of combination effect of hemoglobin (10^{-3} M) and ESEBF (3000 $\mu\text{g/ml}$) on SMA of *P. cervi*

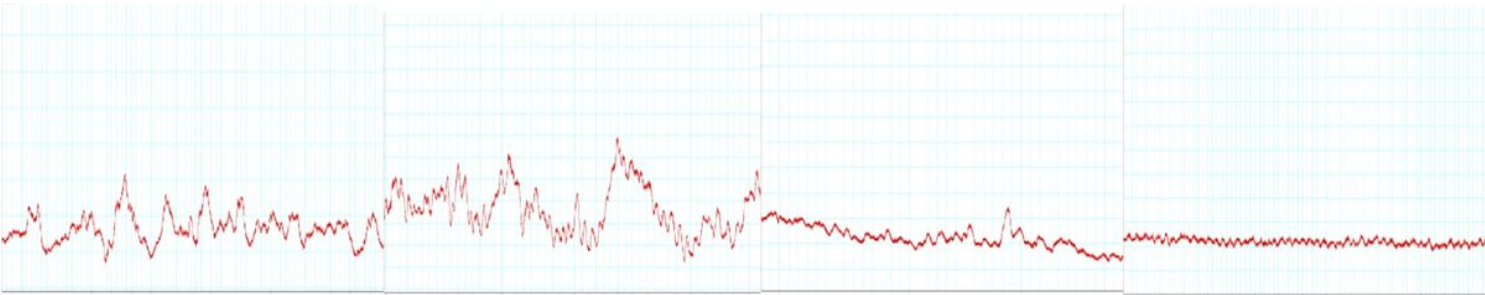


Figure 9

Combination effect of hemoglobin (10^{-3} M) and ESEBF (3000 $\mu\text{g/ml}$) on SMA of *P. cervi*