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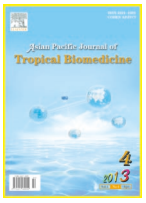

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Antipyretic and anticonvulsant activity of *n*-hexane fraction of *Viola betonicifolia*

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PEER REVIEW

Peer reviewer

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Comments

The authors have reported very interesting data as the plant is used in traditional system of medicines as antipyretic and anticonvulsant so these findings provide strong scientific backup to the folklore of *V. betonicifolia*.
(Details on Page 283)

ABSTRACT

Objective: To investigate the antipyretic and anticonvulsant activities of *n*-hexane fraction of *Viola betonicifolia* (*V. betonicifolia*). **Methods:** The antipyretic effect was scrutinized using brewer's yeast induced pyrexia and anticonvlsion effect was tested using pentylenetetrazol and strychnine induced convulsion in mice. **Results:** *N*-hexane fraction of *V. betonicifolia* demonstrated highly significant antipyretic activity during various assessment times (1-5 h) when challenged in yeast induced pyrexia test. The effect was in a dose dependent manner with maximum attenuation (82.50%) observed at 300 mg/kg *i.p.* When tested in pentylenetetrazol induced convulsion test, the 1st stage (Ear and facial twitching) and 2nd stage (Convulsive wave through the body) was 100% protected during 24 h at all the test doses (300, 400 and 500 mg/kg *i.p.*), while the latency time of remaining stages was significantly increased. The maximum effect was observed by *n*-hexane fraction of *V. betonicifolia* at 400 and 500 mg/kg *i.p.*, as the latency time for generalized clonic-tonic seizure (5th stage) was increased up to 25.34 min. However, *n*-hexane fraction of *V. betonicifolia* had no protection in strychnine induced convulsion test. **Conclusions:** In conclusion, phytopharmacological studies provide scientific foundation to the folk uses of the plant in the treatment of pyrexia and neurological disorders.

KEYWORDS

Viola betonicifolia, *N*-hexane fraction, Antipyretic, Anticonvulsant

1. Introduction

Viola betonicifolia (*V. betonicifolia*) belongs to family Violaceace locally which is known as banafsha. It is found naturally in various countries of the world like Pakistan, India, Nepal, Sri Lanka, China, Malaysia and Australia. In Pakistan, it is available in Swat, Hazara and Dir districts[1]. The folk use of this plant is purgative, antipyretic, astringent, diaphoretic and anticancer. It has been used in the treatments of various neurological disorders including epilepsy and insomnia[2]. Additionally, it has been used in the treatments of sinusitis, skin and blood disorders and pharyngitis[3]. Roots are used for kidney diseases, pneumonia and bronchitis. Flowers are recommended for the treatments of asthma, cough and colds while leaves are used to treat boils[4].

In our earlier studies, the crude methanolic extract as well as the subsequent solvent fraction of *V. betonicifolia* was tested for various pharmacological activities[5-7]. Regarding the phytochemical profile, the plant contains saponins, flavonoids, tannins, proteins, and phenolic compounds[5,7]. Additionally, a new bioactive cinnamic acid derivative has also been isolated[8]. Recently we have published various pharmacological activities of *n*-hexane fraction of *V. betonicifolia*[6,9]. In the *in vivo* medals, the current study was designed to evaluate antipyretic and anticonvulsant activity of the *n*-hexane fraction of *V. betonicifolia* in yeast induced pyrexia test and pentylenetetrazol and strychnine induced convulsion test.

2. Material and methods

2.1. Plant materials

Whole plant of *V. betonicifolia* was collected from

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Swat, Khyber Pakhtunkhwa, Pakistan, in April 2010. The plant specimen was identified by the Taxonomy Section, Department of Botany, University of Peshawar, and a specimen was deposited there in the herbarium under Voucher No. 6410/Bot.

2.2. Preparation of extract

The collected whole plant (12 kg) was air-dried and powdered. The powder plant material was extracted with methanol at room temperature for 14 d with occasional shaking. The methanolic extract was filtered and concentrated under vacuum at low temperature (45 °C). The resulting crude methanolic extract (22%) was further fractionated into various solvent fractions, such as *n*-hexane, chloroform, ethyl acetate, *n*-butanol and aqueous fractions. The *n*-hexane fraction was used for the investigation of various antipyretic and anticonvulsant effects.

2.3. Experimental animals

BALB/c mice of either sex (20-25 g) were used in current experiments. Animals were purchased from the Pharmacology Section, Department of Pharmacy, University of Peshawar, Peshawar. The animals were maintained in standard laboratory conditions (25 °C) and light/dark cycles, *i.e.* 12/12 h and fed with standard food and water *ad libitum*. The experimental protocols were approved by the ethical committee of the department.

2.4. Brewer's yeast pyrexia test

Antipyretic activity of *n*-hexane fraction of *V. betonicifolia* was evaluated by yeast-induced pyrexia test as described earlier[10]. Normal body temperature of the animals was recorded by inserting a rectal thermometer 3-4 cm deep into the rectum. Digital clinical thermometer (Hartmann, Germany) was used for the measurement of temperature. Pyrexia was induced in rats by injection of 10 mL/kg *s.c.* of 15% suspension of Brewer's yeast (Saccharomyces cerevisiae), and they were kept in their housing cages. Rectal temperature of each rat was measured again after 18 h of yeast injection, as described earlier. Animals that developed a minimum increase of 0.5 °C or more in temperature were selected for the experiment. The prescreened animals served as control were arranged in groups (n=6) and treated with saline (10 mL/kg), extracts (100, 200 and 300 mg/kg), or paracetamol (150 mg/kg), were used as a standard drug[7]. After drug treatment, the rectal temperature of each animal was again recorded at 1-h interval up to 5 h. The resulting data were used for the calculation of percentage reduction in rectal temperature. Antipyretic activity was defined as the ability of test drugs to reverse the induced pyrexia.

2.5. Pentylenetetrazole induced anticonvulsant test

For the assessment of pentylenetetrazole induced anticonvulsant activity, our previously reported method was adopted[11]. Briefly, the test animals were divided into five groups (n=6), group I was treated with normal saline (10 mL/kg), group II was treated with diazepam (7.5 mg/kg *i.p.*), while remaining groups were treated with *n*-hexane fraction of *V. betonicifolia* (300, 400 and 500 mg/kg *i.p*). After 30 min of treatment all animals were injected pentylenetetrazole (80 mg/kg *s.c.*). Each animal was observed for onset and mortality.

2.6. Strychnine induced anticonvulsant test

NMRI mice of either sex (18-25 g) were used for screening of *n*-hexane fraction of *V. betonicifolia* (300, 400 and 500 mg/kg). The animals were divided into various groups (n=6). The animals of group I were injected with normal saline (10 mL/kg *i.p.*), served as negative control. Group II was treated with standard drug (diazepam, 7.5 mg/kg *i.p.*). Similarly, the animals of group III-V were treated with *n*-hexane fraction of *V. betonicifolia* (300, 400 and 500 mg/kg *i.p.*) respectively. After 30 min of the above treatment, animals of all groups were treated with strychnine (4 mg/kg *s.c.*) for the induction of convulsions. All animals were observed for the induction of seizure and mortality.

3. Results

3.1. Effect of *n*-hexane fraction of *V. betonicifolia* in antipyretic test

The *n*-hexane fraction of *V. betonicifolia* markedly (P<0.01) attenuated hyperthermia induced by yeast (Table 1). The antipyretic effect of *n*-hexane fraction of *V. betonicifolia* (300 mg/kg) was started from the 1st h and was remained significant up to 5th h of the post-treatment, while at the dose of 200 mg/kg the antipyretic effect was started after 2nd h of the treatment and was remained significant up to 5th h. A weaker antipyretic effect at the dose 100 mg/kg was also observed. The highest antipyretic effect was observed against 300 mg/kg *i.e.* 82.50% in comparison with paracetamol (150 mg/kg) with 85% antipyretic effect. The percent pyrexia inhibition of all the tested groups is shown in Figure 1. The antipyretic effect of the *n*-hexane fraction of

Table 1
Antipyretic effect of VBHF at 100, 200 and 300 mg/kg *i.p.* in yeast induced pyrexia.

Treatment		Rectal temperature after administration of drug (°C)						
		Normal (A)	After 24h (B)	1h (C1)	2h (C2)	3h (C3)	4h (C4)	5h (C5)
Saline (mL/kg)	10	36.66±0.11	38.92±0.34	38.82±0.21	38.78±0.11	38.68±0.20	38.68±0.20	38.72±0.15
Paracetamol (mg/kg)	150	37.08±0.08	39.46±0.04	38.20±0.01**	37.80±0.03**	37.30±0.02**	37.35±0.28**	37.38±0.04**
	100	37.33±0. 04	38.75±2.01	38.58±2.20	38.15±0.31*	37.90±2.12**	38.06±0.20*	38.08±0.02*
VBHF (mg/kg)	200	36.95±0.01	38.80±2.02	38.50±2.12	37.98±1.02*	37.40±1.11**	37.55±1.13**	37.68±1.71*
	300	37.33±3.56	38.48±1.36	38.08±2.11*	37.80±2.76*	37.50±1.25**	37.60±2.65**	37.61±2.15**

Data are reported as mean±SEM (n=6). The data were analyzed by ANOVA followed by Dunnett's test. *: P<0.05, **: P<0.01.
VBHF=*N*-hexane fraction of *V. betonicifolia*.

Table 2

Anticonvulsant effect of ffect of VBHF on PTZ induced convulsion in mice.

Treatment		1st stage	2nd stage	3rd stage	4th stage	5th stage	% Mortality
Saline (mL/kg)	10	5.54±0.98	7.23±0.22	12.34±0.34	15.65±0.22	19.98±0.67	100.00±0.00
Diazepam (mg/kg)	7.5	–	–	–	–	–	–
	300	–	–	14.12±1.23	17.35±2.45	21.35±1.88	–
VBHF (mg/kg)	400	–	–	15.22±1.10*	17.89±1.65*	22.11±2.86*	–
	500	–	–	18.34±1.66*	19.67±1.23*	25.34±2.54*	–

Values are reported as mean±SEM (n=6). The data were analyzed by ANOVA followed by Dunnett's test. *: P<0.05, **: P<0.01 comparing with the control group. VBHF=*N*-hexane fraction of *V. betonicifolia;* PTZ=pentylenetetrazol.

V. betonicifolia was weaker than paracetamol and was higher than VBHF.

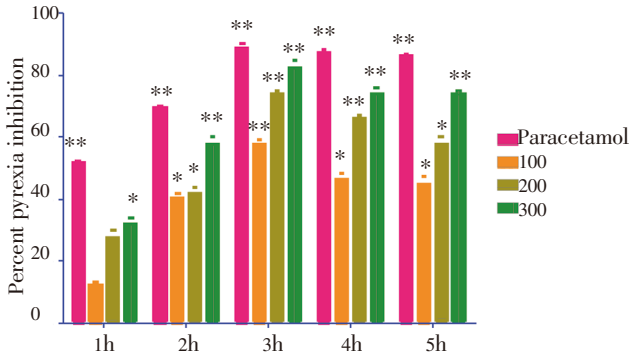

Figure 1. Antipyretic effect of VBHF in mice. Bar presents the percent inhibition of pyrexia after 1, 2, 3, 4 and 5 h of the treatment with paracetamol (150 mg/kg) and VBHF (100, 200 and 300 mg/kg).The data were analyzed by ANOVA followed by Dunnett's test. *: P<0.05, **: P<0.01.

3.2. Effect of n-hexane fraction of V. betonicifolia in pentylenetetrazol induced anticonvulsant test

The anticonvulsant potential of *n*-hexane fraction of *V. betonicifolia* is posted in Table 2. It protected test animals in pentylenetetrazol induced convulsion test at all the stages in comparison to control (10 mg/kg) group. A dose dependant anticonvulsant effect was observed against *n*-hexane fraction of *V. betonicifolia* (300, 400 and 500 mg/kg). The 1st stage (Ear and facial twitching) and 2nd stage (Convulsive wave through the body) was 100% protected at all the test doses, while the latency time of remaining stages was significantly increased. The maximum effect was observed by *n*-hexane fraction of *V. betonicifolia* (400 and 500 mg/kg), as the latency time for generalized clonic-tonic seizure (5th stage) was increased up to 25.34 min. No mortality was observed at any test dose of *n*-hexane fraction of *V. betonicifolia* after 24 h.

3.3.Effect of n-hexane fraction of V. betonicifolia in strychnine induced anticonvulsant test

N-hexane fraction of *V. betonicifolia* did not protect test animals from convulsions at the doses of 300, 400 and 500 mg/kg *i.p.* the animals ultimately died (data is not presented).

4. Discussion

The current research article deals with the antipyretic and anticonvulsant activities of hexane soluble fraction of whole plant of *V. betonicifolia* in established animal protocols. The results reflected profound potential of *n*-hexane fraction of *V. betonicifolia* as antipyretic and protection against pentylenetetrazol provoked convulsions. However, no effects were observed against strychnine induced convulsions.

Subcutaneous injection of Brewer's yeast induces pyrexia by increasing the synthesis of prostaglandin. It is considered as a useful test for the screening of plants materials as well as synthetic drugs for their antipyretic effect[12]. Yeast-induced pyrexia is called pathogenic fever and its etiology could be the production of prostaglandins. The inhibition of prostaglandin synthesis could be the possible mechanism of antipyretic action as that of paracetamol and the inhibition of prostaglandin can be achieved by blocking the cyclo-oxygenase enzyme activity. There are several mediators for pyrexia that regulates the body temperature by interacting with the thermo-regulatory system in the CNS and the inhibition of these mediators is responsible for the antipyretic effect[13].

The intraperitoneal administration of *n*-hexane fraction of *V. betonicifolia* significantly attenuated rectal temperature of yeast induced febrile mice. Thus it can be postulated that *n*-hexane fraction of *V. betonicifolia* contained pharmacologically active principle(s) that interfere with the release of prostaglandins. As a result, they produced antipyretic activity by preventing the formation of prostaglandins or by increasing the concentration of the body's own antipyretic components[14]. Furthermore, the presence of salicylic acids in related species of the genus Viola and the antipyretic action of the *n*-hexane fraction of *Viola odorata* support the antipyretic activity of our tested samples[15]. The present findings provide a strong scientific background to the folkloric use of this plant as antipyretic in the traditional Pakistani medicines.

The convulsion induced by pentylenetetrazol (90 mg/kg) and strychnine (4 mg/kg) are the well known chemical methods used for the induction convulsions in animal models[10,11]. Pentylenetetrazol induce convulsion in animal models due to inhibiting the GABAergic pathway, while the standard anticonvulsant drugs like diazepam enhance the action of GABA[16]. The convulsion induced by strychnine is directly attributed to direct antagonistic effect on reflexes of glycine in the brain and spinal card[17,18]. It is clear from our results that almost all the tested samples antagonised pentylenetetrazol induced convulsion while the strychnine induced convulsion was not antagonized. This give us a clear idea that the *n*-hexane fraction of *V. betonicifolia* acting on the GABAergic mechanism rather than glycine.

In 1972, Racine reported five stages of convulsion, known as Racine's seizure score. According to this seizure score, the 1st stage is ear and facial twitching of mice, 2nd stage is convulsive wave through the body, 3rd stage is myoclonic jerks, 4th stage is clonic-tonic convulsions (the animal turn over into side position) and the 5th stage is generalized clonic-tonic seizure (the animal turn over into back position). A dose dependant anticonvulsant effect was observed by *n*-hexane fraction of *V. betonicifolia* against pentylenetetrazol (90 mg/kg) induced convulsion. It showed 100 % inhibition of

the first two stages of convulsion and the latency time for stage 3 to 5.

GABA is a major inhibitory neurotransmitter in central nervous system (CNS) and epilepsy is the outcome of the inhibition of GABA. There are two types of GABA *i.e.* GABAA and GABAB[19]. The anticonvulsant activity of *n*-hexane fraction of *V. betonicifolia* may be due to stimulation of GABAA receptors. However, further study is needed to find the accurate mechanism of anticonvulsant effect of *n*-hexane fraction of *V. betonicifolia*.

In conclusion, the study showed outstanding antipyretic and anticonvulsant activity of *n*-hexane fraction of *V. betonicifolia* in animal models. Our phytopharmacological studies strengthened the traditional belief and provided scientific background to the uses of the plant in the treatment of pyrexia and neurological disorders.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Comments

Background

This research article has sound background. *V. betonicifolia* is a well kwon medicinal plant having antipyretic and antiepileptic potentials. The author has been reported previously various neuropharmacological priorities of this plant. Therefore, it is very essential to check the *n*-hexane fraction for its antipyretic and anticonvulsant property to provide scientific background to the folklore of *V. betonicifolia*.

Research frontiers

This study has been performed to find the antipyretic and anticonvulsant potential of this plant.

Related reports

The method used for antipyretic and anticonvulsant screening is well recommended and even the author has several research publications on this method, however, no data is reported about the antipyretic and anticonvulsant studies on *n*-hexane fraction of *V. betonicifolia*.

Innovations and breakthroughs

Data regarding the antipyretic and anticonvulsant effect of *n*-hexane fraction of *V. betonicifolia* indicated that the plant is a potential source of antipyretic molecules and is a weak anticonvulsant.

Applications

As the *n*-hexane fraction of *V. betonicifolia* proved good antipyretic and weak anticonvulsant so it is strongly recommended that the plant should be used for these human disorders.

Peer review

The authors have reported very interesting data as the plant is used in traditional system of medicines as antipyretic and anticonvulsant so these findings provide strong scientific backup to the folklore of *V. betonicifolia*.

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