

ASSESSMENT OF *ANEMONE PULSATILLA* FOR SOME BIOLOGICAL ACTIVITIES

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ABSTRACT

Preliminary screening of herbal extracts employs the determination of their biological activity and the estimation of their therapeutic potential. This practice eliminates the unnecessary effort of fractionation and purification of constituents unimportant from the pharmacological point of view. It also gives a scientific basis to the herbs traditionally used in folk medicine. Thus, during the present investigation the spasmolytic effect of *Anemone pulsatilla* extract on isolated tissues of rabbit jejunum was evaluated. In addition the pharmacological screening of *Anemone pulsatilla* extract was also carried out.

INTRODUCTION

Nature's ability to synthesize highly diverse chemicals some of which possess uniquely selective biological activities is well known to today's scientists involved in drug discovery. Among 119 important plant derived drugs used in one or more countries 88 (77%) have been discovered as a result of being derived from a plant used in traditional medicine (Fransworth et al., 1985). An ethnopharmacological approach to the discovery of natural product drugs has gained tremendous importance in recent years.

Anemone pulsatilla, belonging to family Ranunculaceae, has long been used for a wide range of medicinal purposes. *Anemone pulsatilla* is also called pasque flower and is distributed in temperate and Alpine Himalayas, Central Europe and Western Asia (Karnick I., 1994). The herb has a thick and somewhat woody root-stock from which arises a rosette of finely divided, stalked leaves covered with silky hairs. The flowers are borne singly with dull violet-purple sepals, very silky on the under surfaces (Grieve, 1994).

The drug Pulsatilla, a highly valuable modern curative is obtained from the whole herb of *Anemone pulsatilla*. Pulsatilla contains a glycoside ranunculin (Martindale). The fresh plant yields an acrid oily substance called, Oil of Anemone which has a burning peppery taste. This oily substance decomposes into Anemonin and Anemonic acid. Anemonin is a crystalline substance, tasteless and odourless when pure. *Anemone pulsatilla* virtually owes its biological actions to Anemonin. It is a powerful irritant and in overdoses cause violent gastroenteritis. The oil acts as a vesicant when applied to the skin. Anemonic acid seems to be inert. Anemonin may cause local inflammation and gangrene when subcutaneously injected, vomiting and purging when given internally (Grieve, 1994).

The chief action of Anemonin is a depressant one on the circulation, respiration and spinal cord. In poisoning by extract of *Pulsatilla* convulsions are always present.

Anemone pulsatilla is used as a amine, antispasmodic, alterative and diaphoretic. The tincture of *Pulsatilla* is beneficial in disorders of mucous membrane of the respiratory and digestive passages. It is also valuable as an emmenagogue, in the relief of headaches and neuralgia.

Anemone pulsatilla is included in the British Pharmacopoeia and was formerly included in the United States Pharmacopoeia. In Homeopathy it is a weather-cock among remedies (Boericke, 1991). It is prescribed by the herbalists in the treatment of wide range of diseases which include tension, headache, diarrhea, indigestion, bilious attacks, hypertension, insomnia, skin eruptions, bacterial infections, asthma and pulmonary disease.

Specific actions of *Anemone pulsatilla* are sedation, analgesia, spasmolysis and bactericidal (Kariick (II), 1994).

In view of the importance of *Anemone pulsatilla* in traditional medicine, during present investigation, the spasmolytic activity of *Anemone pulsatilla* on the isolated rabbit jejunum was observed. The pharmacological screening was also carried out to determine its effects on the central nervous system.

EXPERIMENTAL

Effect of ethanolic extract of *Anemone pulsatilla* on the spontaneous activity of intestinal smooth muscles (*in vitro* test).

Animals

Locally bred adult male rabbits weighing 2-3 kg were used in this study.

Method

The animals were killed by cervical dislocation and dissected. The jejunum was removed and cut into 2 cm segments. Each segment was mounted vertically under a tension of 1g in a 30ml organ bath filled with Kreb's solution. The organ bath solution was continuously aerated with 95% O₂ and 5% CO₂ gas mixture and thermostatically maintained at 37°C. Spontaneous jejuna) contractions were recorded isometrically using a Grass 7D model Polygraph. The tissues were allowed to equilibrate for 30 minutes. During this period the Kreb's solution in the organ bath was changed after every 15 minutes. This is a cumulative dose-response study. The 100µl ethanolic extract of *Anemone pulsatilla* were added into the organ bath each time until the complete inhibition of the spontaneous activity i.e. 100% relaxation of intestinal smooth muscles.

Pharmacological screening of ethanolic extract of *Anemone pulsatilla* (in vivo test)

Animals

Albino mice, either sex, +NMRI strain, weighing 20-30g, purchased from H.E.J. Research Institute of Chemistry, were used in this study. The animals were given a period of acclimatisation of one week and *ad libitum* access to food and water.

Method

On the day of experiment, the animals were weighed and divided into five groups (n=5). The dose for each animal was calculated according to the weight of that animal (0.2ml/20g). Ethanolic extract of *Anemone pulsatilla* was used in a dose of 50mg/kg. The control group was given the vehicle i.e. water for injection. The doses were administered via injections given intraperitoneally.

The behaviour and physical appearance of the mice were observed immediately after injection for four hours. The observations were made using the guidelines for pharmacological screening (Miranda, 1986) including CNS stimulation, CNS depression, eye and ear observations. The observations were carefully recorded in a multidimensional observation and worksheet. The animals were again checked after 24 and 48 hours for lethal effects and toxicity.

RESULT AND DISCUSSION

The *Anemone pulsatilla* (ethanolic extract) is spasmolytic in action as shown in table 1 and fig. 1. The extract exhibited complete inhibition of the spontaneous activity of isolated rabbit jejunum in a dose of 600 µl, fig. 2. This inhibition is dose dependent and reversible. *Anemone pulsatilla* has long been used in traditional medicine as an antispasmodic. The present study lends credence to its use in diarrhea.

The results of the pharmacological screening of *Anemone pulsatilla* (ethanolic extract) are summarised in table 2. No significant change in the normal behaviour of mice given the dose of 50mg/kg of *Anemone pulsatilla* (ethanolic extract) was observed when compared with the control group. Abdominal stretching was observed in the test animals which is probably due to localised effects of the extract since it has been reported as an irritant (Grieve, 1994). Increased grooming and exploratory behaviour were also observed in the test animals. No lethal effects were observed in the test group after 48 hours.

The traditional use of *Anemone pulsatilla* as a nervine and in the treatment of insomnia, tension and neuralgia calls for studies to determine its exact mode of action by estimating the changes in brain biogenic amines.

Since this is a preliminary study, involving the determination of presence or absence of biological activity on scientific basis, the positive results have paved way for further studies and investigation of this herb as an effective phytoceutical agent

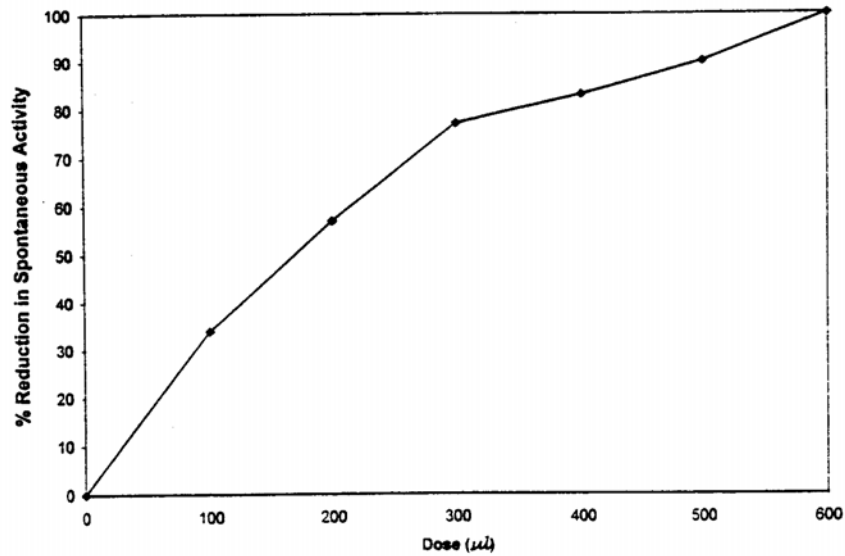


Fig. 1: A tracing showing the spasmolytic activity of *Anemone pulsatilla* (ethanolic extract) on isolated smooth muscles of rabbit jejunum.

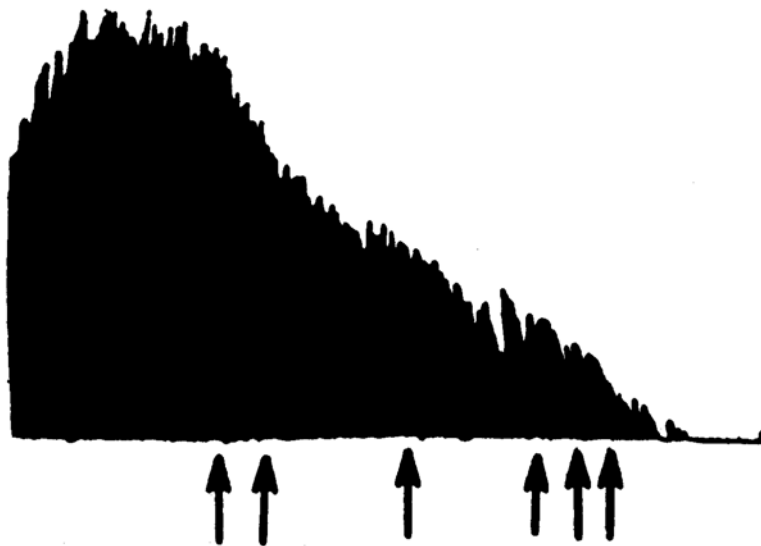


Fig. 2: Cumulative Dose-Response Curve of *Anemone pulsatilla* showing 100% inhibition of the spontaneous activity of isolated smooth muscles of rabbit jejunum.

Table 1
The percentage reduction in the spontaneous activity of isolated smooth muscles of rabbit jejunum by *Anemone pulsatilla* (ethanolic extract)

Cumulative dose (μ l)	% Reduction in Spontaneous activity of isolated smooth muscles
0	0
100	34
200	57
300	77
400	83
500	90
600	100

Table 2
Pharmacological screening of *Anemone pulsatilla* (ethanolic extract)

Sr. No.	Behavioural Parameters	Response	
		Control	Test
a)	CNS Depression		
1	Motor activity	0	0
2	Ataxia/Staggering gait	-	
3	Anacsthsia	-	
4	Respiratory rate	0	0
5	Paralysis	-	-
6	Screen griping	0	0
7	Sedation		-
b)	CNS Stimulation		
1	Startle response	0	0
2	Motor activity	0	0
3	Coarse body tremors	-	-
4	Convulsions	-	
5	Fasciculation	-	-
c)	Eye observation		
1	Pupil size	0	0
2	Nystagmus	-	-
3	Lacrimation	-	
4	Exophthalmos	-	-

Sr. No.	Behavioural Parameters	Response	
		Control	Test
5	Endophthalmos	-	-
d)	Ear observations		
1	Blanching	-	-
2	Hyperemia	-	-
3	Cyanosis	-	-
c)	General observations		
1	Grooming	0	+
2	Irritability	0	0
3	Tail erection	-	
4	Spontaneous activity	0	0
5	Touch response	0	0
6	Sound response	0	0
7	Passivity	-	
8	Writhing	-	-
9	Body tone	0	0
10	Climbing	0	0
11	Stereotype	-	
12	Abdominal stretching	-	+
13	Vocalisation	-	-
14	Salviation	-	
16	Diarrhoea	-	-

0 = No change in normal response

+ = Increase in normal response/Presence of abnormal response

- = Decrease in normal response/Absence of abnormal response

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