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Spasmolytic and spasmogenic activities of crude extract and subsequent fractions of *Paeonia emodi*

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The crude extract and subsequent fractions from the aerial parts of *P. emodi* were studied for their effects on the isolated rabbit jejunum. The crude extract displayed significant spasmolytic activity in a dose-dependent manner and inhibited the spontaneous motility of the rabbit jejunum by 76% at 5 mg/mL concentration. The ethyl acetate and chloroform fractions exhibited excellent spasmolytic activities and were more potent than the native crude extract. The *n*-butanol fraction showed a very low inhibitory activity in this bioassay. The water soluble fraction, unlike crude extract, displayed an overall spasmogenic activity. The crude extracts also effectively reduced the acetylcholine induced contractions in the isolated rabbit jejunum.

Paeonia emodi Wall. (Paeoniaceae), is distributed in Pakistan, India, Nepal and China (De-yuan 2004). Various parts of this plant are used in traditional medicine to cure backache, dropsy, epilepsy, headache, dizziness and is also a tonic, emetic, cathartic, blood purifier, colic, purgative and a pregnancy aid (Shinwari et al. 2003; Hamayun, et al. 2004; Ahmad and Sher 2004). Phytochemical reports have shown that the main constituents of *P. emodi* are 1β, 3β,5α,23,24-pentahydroxy-30-12,20(29)-dien-28-oic acid, oleanolic acid, betulinic acid, ethyl gallate, methyl grevillate, 1,5-dihydroxy-3-methylanthraquinone (Nawaz et al. 2000), wurdin, benzoylwurdin, paeoniflorin, lactiflorin, oxypaeoniflorin (Muhammad et al., 1999), emodinol, benzoic acid, 3-hydroxybenzoic acid (Riaz et al. 2003a), paeonins A and B (Riaz et al. 2003b).

In our previous investigations, extract and fractions from aerial parts of *P. emodi* were found to possess significant enzyme inhibition and radical scavenging activities (Khan et al. 2005a), phytotoxicity, heamagglutination and insecticidal activities with no brine shrimp cytotoxicity (Khan et al. 2005b). In view of the traditional uses of *P. emodi* in various gastrointestinal disorders, the extract from aerial parts and subsequent fractions were tested on isolated intestinal preparations to rationalize its uses in folk medicines.

The crude extract of *P. emodi* displayed a significant spasmolytic activity on the isolated rabbit jejunum in a dose-dependent manner in the concentration range of 0.5–5.0 mg/mL up to 76.00 ± 2.52% inhibition of jejunum contractions (Table).

Table: Spasmolytic and spasmogenic activity of crude extract and subsequent fractions of *P. emodi* in isolated rabbit's jejunum

Extract/fraction	Dose* (mg/mL)	Inhibition/stimulation of contractions** (%)
Crude extract	0.5	31.75 ± 2.65
	1.0	37.38 ± 2.28
	2.0	56.43 ± 6.79
	5.0	76.00 ± 2.52
Ethyl acetate	0.165	5.12 ± 1.34
	0.333	68.05 ± 4.28
	0.666	77.32 ± 3.55
	1.0	100
Chloroform	0.333	5.53 ± 1.52
	0.666	42.05 ± 5.38
	1.0	57.24 ± 3.55
	2.0	70.28 ± 5.34
n-Butanol	0.5	<10
	0.666	<10
	1.0	<10
	2.0	22.16 ± 5.33
Water	0.333	16.35 ± 4.8
	0.666	80.9 ± 5.0
	1.0	42.58 ± 3.76
	2.0	28.99 ± 1.41

* The doses were introduced in individual method and represent the final bath concentrations
** Stimulation for water fraction and inhibition for the rest of fractions and crude extract

The ethyl acetate fraction exhibited a tremendous spasmolytic activity in a dose-dependent fashion and was found to be more potent than the native crude extract (Table). At a dose of 0.165 mg/mL only a small inhibitory activity (5.12 ± 1.34%) was observed for this fraction. However, when the final bath concentration was increased a significant activity increase occurred. At 1.0 mg/mL dose level the jejunum contractile activity ceased completely. Similarly, the chloroform fraction also showed a dose-dependent spasmolytic activity (Table). This fraction caused 5.53 ± 1.52% inhibition of the rabbit jejunum contractions at the dose level of 0.333 mg/mL,which increased up to 70.28 ± 5.34% at a dose level of 2.0 mg/mL. Although the exact chemical constituents of the ethyl acetate and chloroform fractions of aerial parts of *P. emodi* are unknown it is assumed according to previous phytochemical studies of other parts of this plant that terpenes were the abundant class of compounds in fractions of these or similar polarities (Nawaz et al. 2000; Muhammad et al. 1999; Riaz et al. 2003a; b).

The n-butanol fraction of the crude extract exhibited a very low spasmolytic activity as compared to the native crude extract (Table). This activity was below 10% at final bath concentration of 1.0 mg/mL or less. Only 22.16 ± 5.33% inhibition of jejunum contractions was observed at 2.0 mg/mL dose.

The results obtained for the water soluble fraction (Table) were interesting in a sense that unlike the native crude extract, this fraction displayed an overall spasmogenic activity. A further interesting phenomenon was observed when the results were analyzed with the increase in the dose level. A drastic increase in spontaneous response from 16.35 ± 4.82 to 80.95 ± 5.06 % was seen when the dose was increased from 0.333 to 0.666 mg/mL. However, with further increase in concentration, a declining trend was observed and reached to 42.58 ± 3.76% at 1 mg/mL and to 28.99 ± 1.41% with 2 mg/mL dose. This increase followed by a decrease in spasmogenic activity

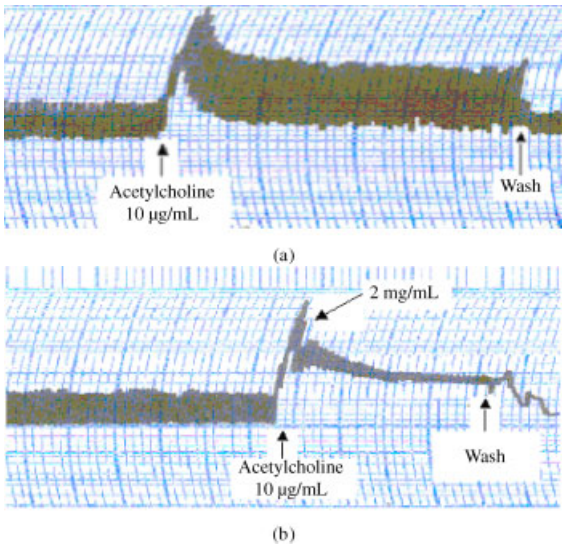

Fig. : Tracings from the typical experiments for effect of crude extracts derived from *P. emodi* on acetylcholine induced spontaneous contractions using isolate jejunum of rabbit, acetylcholine only (a); acetylcholine followed by *P. emodi* extract (b). The doses represent the final bath concentrations

may be due to the presence of both spasmogenic and spasmolytic constituents in this fraction. The spasmolytic constituents, however, may only be effective in higher concentrations and thus overcome the spasmogenic activity when the dose level was increased. This may also be due to some other unknown mechanism. As mentioned above, most of the traditional uses of *P. emodi* are related to the spasmogenic rather than spasmolytic activity. The majority of traditional medicines are taken in decoction and infusion form where water is the solvent. Thus, these preparations may contain the water soluble constituents, which are responsible for the spasmogenic activity of *P. emodi*.

The crude extract of *P. emodi* showed significant spasmolytic activity; therefore it was tested for possible cholinergic activity in the acetylcholine induced contractions in the isolated rabbit jejunum (Fig.). The crude extract significantly reduced the acetylcholine induced contractions and thus may act as spasmolytic agent through inhibition of cholinergic receptors.

This study showed that only the water soluble fraction has spasmogenic activity and thus confirms the traditional uses of *P. emodi* for gastrointestinal disorders. Moreover, the spasmolytic activity of the crude extract is mainly concentrated in the ethyl acetate and chloroform fractions and thus the constituents of these fractions are responsible for the activity of the crude extract. These significant results with ethyl acetate, chloroform and water fractions indicates the need for further work on the isolation and purification of the active principles responsible for spasmolytic or spasmogenic activity.

Experimental

1. Plant material, preparation of extract and fractionation

The plant *P. emodi* (aerial parts) was collected from Swat, Pakistan and identified by Mehboob-ur-Rehman, plant taxonomist, Department of Botany, Government Degree College Matta, Swat. Air-dried and ground plant material was extracted with ethanol for three weeks and subsequently dried under reduced pressure. For fractionation, a part of the crude extract was dispersed in water and successively partitioned with n-hexane, chloroform, ethyl acetate, and n-butanol, as described previously (Khan et al. 2005a)

2. Spasmogenic and spasmolytic activities

Spasmogenic and spasmolytic activities of the extract and fractions from *P. emodi* were evaluated using isolated rabbit jejunum (Gilani et al. 2000). The animals were killed with a blow on the head followed by exsanguinations. Segments of jejunum were mounted in oxygenated Tyrode's solution maintained at 37 °C in an organ bath and allowed to equilibrate for at least 30 min. The crude extract or fractions were dissolved in 2–3 mL of purified water and added to the organ bath following individual dose method. All experiments were performed in triplicate ($n = 3$) and the results were expressed as mean ± S.E.M. The following formula was used for calculations:

$$\text{Inhibition/stimulation (\%)} = 100 - \frac{\text{Average height of contractions after addition of extract (mm)}}{\text{Average height of normal contractions (mm)}} \times 100$$

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