



In-vitro investigations of P-28 an ayurvedic menstrual corrective on the uterine and other smooth muscles

INTRODUCTION

Ayurvedic medicine describes a number of botanical drugs to be useful in menstrual disorders including dysmenorrhoea, dysfunctional uterine bleeding, and irregular cycles amongst others (1, 2, 3). *Saraca indica* (Asoca) bark in India has been used as a uterine sedative, emmenagogue, for treating uterine disorders, menorrhagia as well as used in several preparations related to menstrual disorders (4, 5, 6, 7). Ayurveda recommends bark of *Symplocos racemosa* (Lodhra) in conditions with increased discharge like leucorrhoea and excessive menstrual bleeding amongst others (1). Similarly root bark of *Abroma augusta* is recommended as a valuable emmenagogue and uterine tonic (8). *Asparagus racemosa* (Shatavari) has been shown to have anti-oxytotic action on uterine muscle (9, 10). P-28 is a compound preparation consisting of such herbal drugs recommended by traditional medicine in the treatment of menstrual disorders. This basic pharmacological study was therefore undertaken to assess the effect of this compound preparation on the contractile response of various known spasmogens on isolated smooth muscle preparations including uterine muscles from different species.

METHODS

Preparation of test drug material

Test drug P-28 was supplied by the manufacturers M/s Pharmaveda as a 20 mesh coarse powder blend. Each 850 grams of the test drug P-28 blend contained Ashoka (*Saraca indica*) 300 gms, Ulatkambal (*Abroma*



augusta) 100 gms, Dashmooli (Ten roots) 100 gms, Lodhra (Symlocos racemosa) 100 gms, Motha (Cypreus rotundus) 100 gms, Satavari (Asparagus racemosus) 100 gms and Ashwagandha (Withania somnifera) 25 gms. The dried powdered plant material (1kg) was extracted with 8 liters distilled water till the volume of the macerate went to less than two liters. The water from the extract so obtained was evaporated to give a viscous mass. This extract was then reconstituted with distilled water to have a test material solution of 1 mg/ml and used in the experiments. All doses of test drug P-28 were added in a volume of 0.025 ml, 0.05 ml & 0.1 ml per 10 ml organ bath, that is, at dose of 50 & 100 micrograms/ml respectively.

The experiments were made on isolated strips of guinea-pig ileum, rabbit jejunum, guinea-pig vas deferens, non-pregnant rat uterus and non-pregnant guinea-pig uterus as follows:

Isolated guinea-pig ileum

Male guinea-pigs, weighing between 250 and 300g were used. The animals were stunned and the abdomen was opened, an ileum specimen was removed and mesentery was dissected off and washed with Tyrode's solution. A piece (2 cm) of the terminal ileum was suspended in an organ bath containing oxygenated Tyrode solution at room temperature bubbled with oxygen 95%. The contractions were recorded on a kymograph through a frontal lever. This lever, which magnified the muscular movement, applied a load of 0.5 to 2 g. to the muscle. After being placed in the organ bath, the isolated muscle strips were left for a 60 min. period in order to get used to their experimental environment. During that period the bath solution was renewed at 15



min. intervals. The spasmogens were applied for periods of 3 min, followed by wash-out periods of 4-8 min according to the speed of relaxation of the particular preparation. The spasmolytic activity of the test compound P-28 (0.5 ml) was evaluated by its ability to inhibit the spasm induced by a submaximal concentration (in g/ml) of spasmogens, namely, acetylcholine chloride (2.5×10^{-8}), histamine dihydrochloride (3×10^{-8}), 5-hydroxytryptamine creatine sulphate (5-HT, 5×10^{-7}) and prostaglandin F2 alpha (PGF2alpha, 2×10^{-8}). The spasmogens were applied for periods of 3 min, followed by wash-out periods of 4-8 min according to the speed of relaxation of the particular preparation.

Isolated rabbit jejunum

White New Zealand rabbits (3 kg body weight) were killed and 2 cm segments of the jejunum were cut and used. Pieces of rabbit jejunum (3 - 4 cm) were also suspended in Tyrode solution in a similar organ bath bubbled with oxygen 95% and the movements recorded as in the case of guinea pig ileum. The spasmolytic activity of the test compound P-28 was evaluated similar to the guinea-pig ileum preparation.

Isolated guinea-pig vas deferens

Male guinea-pigs weighing between 250 and 350 g were used. The animals were stunned and the abdomen was opened along the midline and the intestine moved to one side. The vas deferens on each side was cut at one end and the urethra at the other. The mesentery was trimmed off. Each vasa was suspended in organ bath at $37 \pm 0.5^\circ\text{C}$ in Krebs' solution bubbled with oxygen 95%. The spasmolytic activity of the



test compound P-28 was evaluated similar to the guinea-pig ileum preparation.

Isolated non-pregnant Rat uterus.

Virgin female rats weighing between 180 and 220 g were used. The animals were injected subcutaneously with stilbestrol (1 mg/kg) dissolved in olive oil. The animals were stunned and bled 24 h after this treatment. The abdomen was opened and the two horns of the uterus were separated and freed from fat. Each horn was mounted in De Jalon's solution bubbled with oxygen 95% at room temperature. The spasmolytic activity of the test compound P-28 was evaluated similar to the guinea-pig ileum preparation.

Isolated non-pregnant guinea-pig uterus

Nulliparous guinea-pigs of weight 400-490 g, not in estrous (since the spontaneous contractions which occurred at and near oestrous make it difficult to evaluate the effects of stimulants) were used. The animals were stunned by a blow on the neck. The uterus was removed, the horns were divided into two pieces and strips 2 cm long were suspended in Tyrode's solution at room temperature bubbled with oxygen 95%. The spasmolytic activity of the test compound P-28 was evaluated similar to the guinea-pig ileum preparation.

Statistical analysis

Data are presented as mean \pm S.E.M. and Student's t-test was used for comparison of difference between treatments.



RESULTS

Effect of P-28 on the isolated guinea-pig ileum

P-28 by itself had no effect on the isolated guinea-pig ileum, nor did it alter the contractile response at any dose level used to contractions induced by any of the spasmogens used namely, acetylcholine, histamine, 5-hydroxytryptamine or prostaglandin F₂alpha

Effect of P-28 on the isolated rabbit jejunum

P-28 alone had no effect on the isolated rabbit jejunum, nor did it alter the contractile response at any dose level used to contractions induced by any of the spasmogens used namely, acetylcholine, histamine, 5-hydroxytryptamine or prostaglandin F₂alpha

Effect of P-28 on the isolated guinea-pig vas deferens

In the isolated guinea-pig vas deferens, P-28 at doses of up to 100 microgram/ml had no effect alone. P-28 in the doses used also did not diminish or enhance the contractions induced by acetylcholine, histamine, 5-hydroxytryptamine or prostaglandin F₂alpha

Effect of P-28 on the isolated rat uterus

In the isolated rat uterus, P-28 produced a concentration linked reversible inhibition of contractions induced by acetylcholine, histamine, 5-hydroxytryptamine or prostaglandin F₂alpha. The pattern of anti-spasmodic effect of P-28 on the uterine spasms was similar against all the spasmogens. The effect of acetylcholine, histamine, 5-hydroxytryptamine or prostaglandin F₂alpha was more suppressed with



increasing doses of P-28; 25, 50 and 100 microgram/ml, respectively. The mean percentage inhibitions are shown in Table 1.

Effect of P-28 on the isolated guinea-pig uterus

The results of P-28 on the isolated guinea-pig uterus were very similar to those obtained in isolated rat uterus. P-28 produced a concentration dependant reversible inhibition of contractions induced by acetylcholine, histamine, 5-hydroxytryptamine or prostaglandin F₂alpha. The pattern of anti-spasmodic effect of P-28 on the uterine spasms was similar against all the spasmogens. The effect of acetylcholine, histamine, 5-hydroxytryptamine or prostaglandin F₂alpha was more suppressed with increasing doses of P-28; 25, 50 and 100 microgram/ml, respectively. The mean percentage inhibitions are shown in Table 2.

REMARKS

The fact that various drugs can cause a spasm in smooth muscle tissue labels them all as spasmogens. However it needs to be borne that spasmogens are from varied, different and distinct pharmacological, stereo-chemical and chemical groupings - a truly heterogeneous group wherein each spasmogen induces smooth muscle contractions in a distinct and well defined manner. Similarly the evolutionary origin and development of each tissue is distinct. While smooth muscles as a group have a number of common attributes, each smooth muscle tissue due to its evolutionary distinction have developed into distinct quasi types, each with distinct and specific resident receptors & enzymes preponderances.



The test drug in present study, P-28, which is a poly herbal composition of individual herbal drugs mentioned in the authoritative texts of ayurveda to be useful in the treatment of varied menstrual origin, has not shown any effect on the contractions induced on isolated smooth muscle preparations from different species by different classes of spasmogens other than those in the uterine smooth muscles. In the uterine smooth muscle preparations however, both in the isolated rat uterus and the isolated guinea-pig uterus, the test drug P-28 has shown antagonism to the contractile response of all the spasmogens from different classes at all doses used. The utero-specific or uterotropic anti-spasmodic effect of P-28 was dose linked and reversible, with enhancement in the anti-spasmodic effect with increasing dose of P-28 and validates its use in spasmodic uterine disorders like primary dysmenorrhoea.

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Effect of P-28 on Ach, Histamine, 5-HT & PGF2alfa induced contraction in rat-uterus preparations

Drug	Pretreatment control	Inhibitory effect of P-28 at various concentrations (microgram/ml) post-treatment		
		25	50	100
Ach	3.79 ± 0.30 (0)	2.38 ± 0.025 (37.20 ± 2.96)	1.99 ± 0.115 (47.49 ± 1.00)	0.82 ± 0.073 (78.36 ± 2.23)
Histamine	3.40 ± 0.23 (0)	2.54 ± 0.105 (25.29 ± 2.04)	2.03 ± 0.253 (40.29 ± 3.00)	1.05 ± 0.084 (69.11 ± 1.08)
5-HT	3.60 ± 0.189 (0)	2.72 ± 0.285 (24.44 ± 1.89)	2.05 ± 0.101 (43.06 ± 1.54)	1.22 ± 0.143 (66.11 ± 3.00)
PGF2alfa	4.58 ± 0.235 (0)	3.64 ± 0.175 (20.52 ± 1.09)	3.07 ± 0.263 (32.94 ± 3.31)	1.98 ± 0.201 (56.77 ± 2.47)

Acetylcholine chloride (Ach, 2.5×10^{-8}), histamine dihydrochloride (Hist, 3×10^{-8}), 5-hydroxytryptamine creatine sulphate (5-HT, 5×10^{-7}) and prostaglandin F2 alpha (PGF2alfa, 2×10^{-8}) was added in the organ bath.

The values in parentheses represent the inhibitory effect of test drug P-28 as percentage compared with the effect of drug alone (pretreatment). Readings are mean ± S.E.M. of five observations.

All values are significant in comparison with control (pretreatment), $p < 0.001$ (Students t-test).

Effect of P-28 on Ach, Histamine, 5-HT, & PGF2alfa induced contraction in guinea-pigs uterus preparations

Drug	Pretreatment control	Inhibitory effect of P-28 at various concentrations (microgram/ml) post-treatment		
		25	50	100
Ach	2.88 ± 0.30 (0)	2.05 ± 0.025 (28.82 \pm 1.78)	1.89 ± 0.115 (34.38 \pm 1.35)	1.32 ± 0.073 (54.16 \pm 2.05)
Histamine	2.69 ± 0.23 (0)	2.04 ± 0.105 (24.16 \pm 1.90)	1.76 ± 0.253 (34.57 \pm 2.48)	1.10 ± 0.084 (59.11 \pm 2.02)
5-HT	2.60 ± 0.189 (0)	2.01 ± 0.285 (22.69 \pm 1.63)	1.72 ± 0.130 (33.84 \pm 1.72)	1.17 ± 0.143 (55.00 \pm 1.88)
PGF2alfa	3.78 ± 0.235 (0)	2.88 ± 0.175 (23.81 \pm 1.23)	2.27 ± 0.263 (39.95 \pm 2.77)	1.65 ± 0.201 (56.34 \pm 1.93)

Acetylcholine chloride (Ach, 2.5×10^{-8}), histamine dihydrochloride (Hist, 3×10^{-8}), 5-hydroxytryptamine creatine sulphate (5-HT, 5×10^{-7}) and prostaglandin F2 alfa (PGF2alfa, 2×10^{-8}) was added in the organ bath.

The values in parentheses represent the inhibitory effect of test drug P-28 as percentage compared with the effect of drug alone (pretreatment). Readings are mean \pm S.E.M. of eight observations.

All values are significant in comparison with control (pretreatment), $p < 0.001$ (Students t-test).



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