JPP Journal of Pharmacy and Pharmacology

JPP 2008, 60: 1643–1650

Received March 18, 2008
Accepted August 15, 2008
DOI 10.1211/jpp/60.12.0010
ISSN 0022-3573

Depressant-like effects of parthenolide in a rodent behavioural antidepressant test battery

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Abstract

The anti-serotonergic effects of parthenolide (PTL) demonstrated in platelets inspired the present psychopharmacological investigation, which employs a battery of rodent behavioural assays of depression. In mice, PTL (0.5–2 mg kg^{-1}) exhibited dose-dependent depressant-like effects in a forced swim test and a tail suspension test, without affecting the baseline locomotor status. The doses (1 and 2 mg kg^{-1}) that induced depressant-like effects were found to significantly reduce 5-hydroxytryptophan-induced head twitch response. Interaction studies revealed that the depressant-like effects of PTL (1 mg kg^{-1}) were reversed more efficiently by serotonergic antidepressants (venlafaxine, escitalopram, citalopram, fluoxetine) than by others (desipramine, bupropion) tested. Chronic treatment of PTL (1 and 2 mg kg^{-1}) augmented the hyper-emotionality of olfactory bulbectomized rats, when compared with sham rats, as observed in modified open field, elevated plus maze and social interaction paradigms. This study depicts the severe depressogenic potential of PTL (in its pure form) plausibly mediated by platelet/neuronal hypo-serotonergic effects.

Introduction

Parthenolide (PTL) is a germacranolide sesqueterpene lactone isolated from different species of plants of the Asteraceae (Compositae) family, one of them being feverfew (*Tanacetum parthenium*). Its chemical structure was elucidated in the mid 1960s (Govindachari et al 1964, 1965) and investigations (as described below) identified interesting pharmacological properties. The percentage of PTL in feverfew plays a pivotal role not only in its anti-migraine effects (Gromek et al 1991) but also in the component mechanisms, such as inhibition of 5-hydroxytrypamine (5-HT) release (Heptinstall et al 1992; Marles et al 1992) and 5-HT inhibitory activity in isolated rat fundus and ileum (Mittra et al 2000). The probable actions of PTL on central nervous system (CNS) receptors and inhibition of serotonin release accentuates the requirement of in-vivo behavioural data on this molecule. In-vitro studies of 5-HT release from platelets demonstrated that PTL inhibits 5-HT secretion and platelet (human) aggregation mediated via the protein kinase C pathway (Groenewegen & Heptinstall 1990) and such an inhibition was dependent on platelet activating factor in equine platelets (Bailey et al 2000). PTL inhibited the fenfluramine and dextroamphetamine (indirect acting serotonergics) mediated 5-HT release in isolated rat fundus (Béjar 1996). Binding studies indicated that PTL had no affinity for 5-HT_{1A} receptors (Weber et al 1997a) but exhibited some antagonistic effects at 5-HT_{2A} receptors (Weber et al 1997b). The log P value of PTL was reported as 2.4 (Wahlkvist et al 2008), indicating a highly probable blood–brain barrier (BBB) permeability. Few pharmacological and clinical studies have reported its central actions, the most notable being its anti-migraine effect (Murphy et al 1988; Awang 1998; Tassorelli et al 2005), and one of the proposed mechanisms behind such an effect is the inhibitory actions of PTL on serotonin release (Marles et al 1992).

Animal models of depression have been utilized vigorously to screen novel compounds (Bourin 1990) and were originally designed as screening tests to assess the efficacy of antidepressant drugs. These tests neglect the aspect of face validity but have a strong predictive validity to aid in the identification of efficient antidepressant substances (Lucki 1997). Hence we utilised a battery of behavioural tests – forced swim test (FST) (Porsolt et al 1977), tail suspension test (TST) (Borsini & Meli 1988), effects on 5-hydroxytryptophan (5-HTP) induced head twitch response (HTR) and olfactory bulbectomized (OBX) rats – to provide significant

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Acknowledgments: The authors wish to thank Glenmark Pharmaceuticals Ltd, IPCA Laboratories Ltd and Ranbaxy Research Laboratories, India for the generous provision of conventional antidepressants, at short notice.

information on PTL-induced modulation of depression-related behaviour in rodents. To the best of our knowledge, we report the first investigation that has employed whole-animal assays to probe the psychopharmacological properties of this lactone.

Materials and Methods

Drugs

PTL was obtained from Tocris chemicals (UK). Desipramine hydrochloride, pargyline and 5-HTP were procured from Sigma Chemicals (USA). Escitalopram oxalate was obtained as a gift sample from Glenmark Pharmaceuticals Ltd (India); fluoxetine hydrochloride, paroxetine hydrochloride and citalopram hydrobromide were gifted by IPCA laboratories (India); bupropion and venlafaxine were gifted by Ranbaxy Research Laboratories (India). All other chemicals used were of analytical grade.

Animals

Male Swiss albino mice, 25–35 g, and Wistar albino rats, 225–300 g, were obtained from Hissar Agricultural University (Hissar, Haryana, India) and maintained under conditions of standard lighting (lights on: 0700–1900 h), temperature (23 ± 2°C) and room humidity (60 ± 10%). They were housed in standard plastic cages and provided with free access to food (standard pellet chow) and filtered water. Experimental sessions began after 20 days' quarantine period. The animals were used only once for each experiment and strictly acclimatized to the experimental room for 1 h before testing. Experiments on animals were approved by the Institutional Animal Ethics Committee of Birla Institute of Technology & Science, Pilani, India (Protocol No.IAEC/RES/4/1, dated 22.09.04; IAEC/RES/7/1, dated 24.04.06).

Methodology

The animals were randomized into control and experimental groups (n = 8). All drugs were dissolved in deionized water and administered per-orally, with the exception of PTL which was dissolved in 5% ethanol and administered intraperitoneally. Vehicle groups received 5% ethanol in deionized water.

Dose–response studies

Dose–response studies were carried out using the mouse spontaneous locomotor activity (SLA) test FST and TST, to determine the appropriate doses of PTL that significantly influenced the depressive state without affecting the baseline locomotor status. The PTL (0.25–2 mg kg^{-1}, i.p.) was administered to mice 30 min before subjecting them to assessment of spontaneous locomotor activity (SLA) or in forced swim test (FST)/tail suspension test (TST). Selected doses were used for the confirmatory and interaction studies.

Confirmatory studies

Confirmatory studies included the assessment of effects of PTL (1 and 2 mg kg^{-1}) on 5-HTP-induced HTR in mice and on the behaviour of OBX rats in modified open-field, elevated plus maze and social interaction. In the 5-HTP study, mice were treated with PTL (1 or 2 mg kg^{-1}); 30 min after PTL administration the mice then received pargyline (100 mg kg^{-1}) and 5-HTP (5 mg kg^{-1}) 30 min and 15 min before observation, respectively.

In the OBX rat model of depression, after a post-surgical rehabilitation period of 14 days, the OBX rats received PTL (1 and 2 mg kg^{-1}), paroxetine (10 mg kg^{-1}) or vehicle, once a day for 14 days (15th to 28th day). All administrations and testing were done between 10–14 h. On the 29th day, the OBX rats were then subjected to open field (20 h after the last dose) and followed by elevated plus maze exploration paradigm on the 30th day. On the 31st day the rats were assessed for social interaction behaviour (Table 1). To avoid bias, behavioural assays were performed by experimenters who were blind to the treatment of each animal.

Interaction studies

The interaction study with marketed antidepressants was carried out in mice using the FST. Mice were treated individually with a single dose (p.o.) of vehicle, escitalopram (10 mg kg^{-1}), venlafaxine (5 mg kg^{-1}), citalopram (20 mg kg^{-1}), fluoxetine (20 mg kg^{-1}), desipramine (20 mg kg^{-1}) or bupropion (20 mg kg^{-1}). The doses of standard antidepressants were obtained from pilot studies or from previous studies carried out in our laboratory (Mahesh et al 2007; Ramamoorthy et al 2008). Thirty minutes later, all the mice received PTL (1 mg kg^{-1}, i.p.). Thirty minutes after the PTL injection, mice were subjected to FST/TST. All the equipment used to assess the rodent's behaviour was sprayed with alcohol and wiped thoroughly between trials to eliminate the residual odour.

Behavioural assays

Spontaneous locomotor activity (SLA)

The SLA was assessed in mice as per procedure mentioned elsewhere (Boissier & Simon 1965) with slight modifications using an actophotometer. The mice were individually placed in a square arena (30 cm × 30 cm), walls painted

Table 1 Surgery and treatment schedule to assess the effect of parthenolide on olfactory bulbectomized rats

Surgery and treatment				**Behavioral assessment (20 h after last drug treatment)**		
Day 0	Day 0–1	Day 1–14	Day 15–28	Day 29	Day 30	Day 31
Bilateral olfactory bulbectomy	Recovery from surgery (continuous care)	Rehabilitation period (daily handling and observation)	Vehicle/drug/test compound treatment (i.p. administration, once a day for 14 days)	Modified open field exploration	Elevated plus maze exploration	Social interaction paradigm

black and with photocell assembly. After an initial 2-min familiarization period, the digital locomotor scores were recorded for the next 10 min in a dimly lit room.

Forced swim test

The FST was slightly modified (Mahesh et al 2007) from the originally described (Porsolt et al 1977) method. In brief, each mouse was placed individually in a glass cylinder (diameter 22.5 cm, height 30 cm) filled with water at a height of 15 cm. The floor of the cylinder was demarcated in to four equal quadrants. The mice were forced to swim for 15 min on the 1st day. Mice were then allowed to return to their home cage. On the 2nd day, each mouse (vehicle/drug treated) was placed again into the water and forced to swim for 6 min. The duration of immobility and number of quadrants crossed during the last 4 min was measured. The mouse was considered as immobile when it stopped struggling and passively moved to remain floating to keeping its head above water. Water was changed between trials and temperature was maintained at 22–23°C.

Tail suspension test

Behavioural despair was induced by tail suspension according to the procedure of Steru et al (1985) with slight modifications (Mahesh et al 2007). Mice were suspended individually using an adhesive tape from a horizontal bar 50 cm above the flat surface of the tabletop. The point of attachment on the tail was 1 cm from the tip. The duration of immobility during the 6-min observation period was recorded. Mice were considered immobile only when they were completely motionless. The parameter recorded was the number of seconds spent immobile.

5-Hydroxytryptophan-induced head twitch response

The method mentioned elsewhere (Martin et al 1989) was adopted with slight modifications. Fifteen minutes after 5-HTP (5 mg kg^{-1}) administration, the number of head twitches exhibited by the mice (vehicle or drug treated) during the next 15 min was recorded as head twitch scores. The head twitch response was characterized by abrupt lateral movements, which may be accompanied by body twitches and hind limb retraction. The mice were then rehabilitated after completion of the study.

Rat olfactory bulbectomy

Bilateral olfactory bulbectomy was performed as described earlier (Mahesh et al 2007; Ramamoorthy et al 2008) with slight modification as mentioned below. Briefly, the rats were anaesthetized with xylazine (5 mg kg^{-1}) and ketamine (75 mg kg^{-1}, i.p.). The head of the rat was fixed in a stereotaxic frame and the skull was exposed by a midline sagittal incision. Burr holes (2 mm in diameter) were drilled 8 mm anterior to bregma and 2 mm on either side of the midline at a point corresponding to the posterior margin of the orbit of the eye. The olfactory bulbs were removed by suction, the holes were then filled with haemostatic sponge to control excessive bleeding and the scalp was sutured. To prevent post-surgical infection, the rats were given Sulprim injection (each mL containing 200 mg of sulfadiazine and 40 mg of trimethoprim) intramuscularly (0.2 mL/300 g) once a day for 3 days, post-surgery. Sham-operated rats were treated in the same way, including piercing of the dura mater, but their bulbs were left intact.

Open field exploration

The OBX and sham rats were subjected to an open field test on the 29th day post surgery and the 15th day of chronic drug/vehicle administration. The open field exploration was conducted as described by Kelly et al (1997) with slight modifications. The apparatus consisted of a circular (90 cm diameter) arena with 75-cm-high aluminium walls and a floor equally divided into 10-cm squares. A 60 W light bulb was positioned 90 cm above the base of the arena, which was the only source of illumination in the testing room. Each rat was individually placed in the centre of the open field apparatus and the ambulation scores (number of squares crossed) was noted for 5 min.

Elevated plus maze

The experimental protocol standardized in our laboratory (Ramamoorthy et al 2008) was adopted. The plus-maze consisted of two open arms (50 cm × 10 cm) and two enclosed arms (50 cm × 10 cm × 50 cm). The four arms were joined by a central platform (10 cm × 10 cm) open to all the arms, to form a plus shape. The entire apparatus was elevated to a height of 60 cm above the floor. The apparatus was indirectly illuminated with a ceiling-fronting lamp (60 W), which was placed 100 cm above the apparatus. The test was performed on the 30th day after surgery after exposure to the modified open field. At the beginning of the test, the rat was placed in the central platform facing an open arm. The time that the rat spent in the open arms was recorded for 5 min.

Social interaction test

This protocol was adapted (with slight modifications as mentioned below) from elsewhere (File & Hyde 1978). This test was performed on the 31th day after OBX surgery. The same apparatus and testing environment as that of the open field test, except that the illumination was milder (8 W), was used for the social interaction test. On the day of test, pairs of rats, of the same treatment/surgery group housed in different cages, were put into two different corners of the open field arena. The social interaction behaviour, including the frontward running, probing, grooming, mounting and crawling under the other rat, were recorded for 10 min.

Statistics

All the data were expressed as mean ± s.e.m. The single treatment studies were analysed using one-way analysis of variance followed by Dunnett's T3 test. The interaction studies were analysed using two-way analysis of variance followed by post-hoc Sidak test. The level of statistical significance was fixed at $P < 0.05$.

Results

Spontaneous locomotor activity

The locomotor activity (Figure 1A) of mice was not significantly influenced by PTL (0.25–2 mg kg^{-1}) treatment ($F(4,35) = 0.679$, NS).

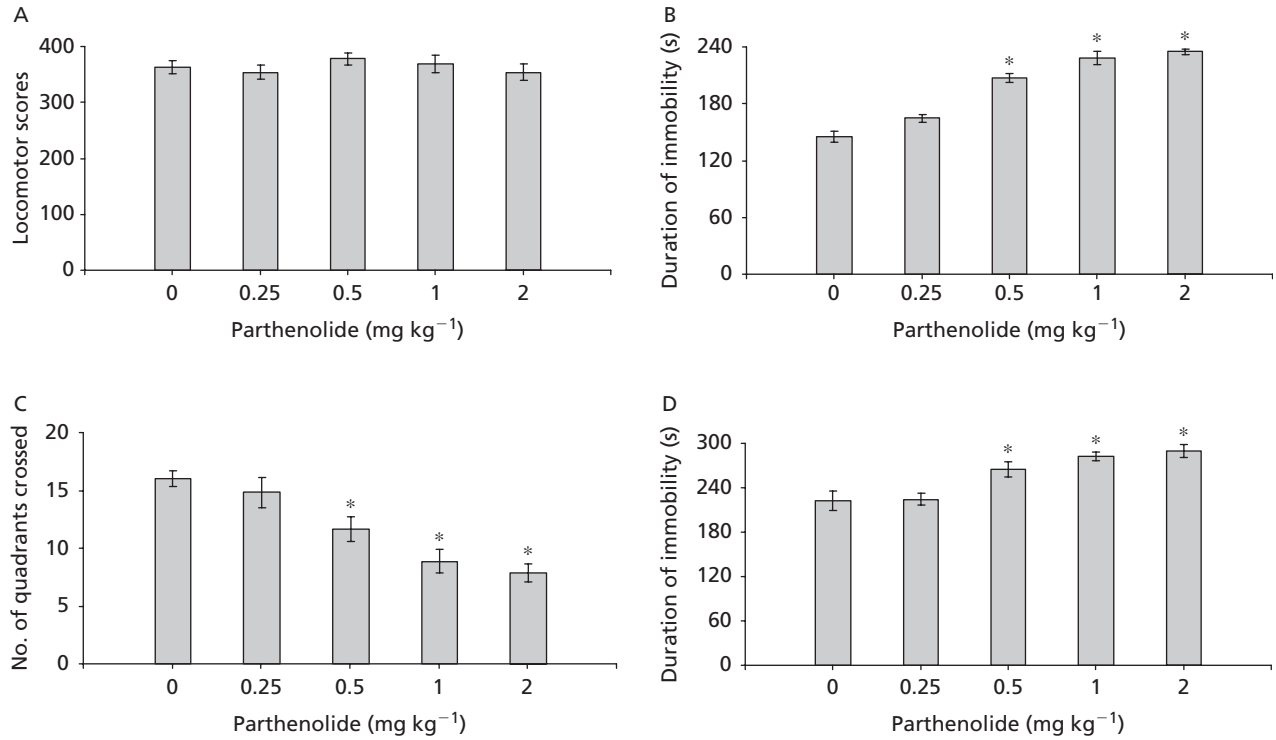

Figure 1 A. Effect of parthenolide on spontaneous locomotor activity of mice. The columns represent mean locomotor scores recorded in a 10-min observation period. The error bars indicate s.e.m., n = 8 per group. B. Effect of parthenolide on duration of immobility of mice in forced swim test. The columns represent mean duration of immobility in seconds (s) and error bars indicate s.e.m., n = 8 per group. *$P < 0.05$ compared with vehicle-treated group (0 value on abscissa). C. Effect of parthenolide on swimming behaviour of mice in forced swim test. The columns represent mean number of quadrants crossed and error bars indicate s.e.m., n = 8 per group. *$P < 0.05$ compared with vehicle-treated group (0 value on abscissa). D. Effect of parthenolide on duration of immobility of mice in tail suspension test. The columns represent mean duration of immobility (s) and error bars indicate s.e.m., n = 8 per group. *$P < 0.05$ compared with vehicle-treated group (0 value on abscissa).

Table 2 Effect of pre-treatment with various antidepressants on parthenolide (PTL)-induced behaviour in mouse forced swim test

Treatment (dose, mg kg^{-1})	Duration of immobility (s)	Swimming episodes
Vehicle	173.67 ± 3.21	18.00 ± 1.09
PTL (1)	234.25 ± 4.08*	8.30 ± 0.89*
Venlafaxine (5)	88.75 ± 5.23*$^{\#}$	37.00 ± 2.04*$^{\#}$
Escitalopram (10)	69.88 ± 8.92*$^{\#}$	41.25 ± 2.09*$^{\#}$
Citalopram (20)	108.25 ± 10.31*$^{\#}$	34.00 ± 2.92*$^{\#}$
Fluoxetine (20)	101.75 ± 8.79*$^{\#}$	34.75 ± 2.74*$^{\#}$
Desipramine (20)	109.75 ± 8.49*$^{\#}$	29.25 ± 2.11*$^{\#}$
Bupropion (20)	133.75 ± 9.73*$^{\#}$	24.00 ± 1.57$^{\#}$
Venlafaxine (5) + PTL (1)	129.25 ± 4.09*$^{\#}$	36.12 ± 2.54*$^{\#}$
Escitalopram (10) + PTL (1)	120.00 ± 5.09*$^{\#}$	40.25 ± 2.63*$^{\#}$
Citalopram (20) + PTL (1)	152.88 ± 3.03*$^{\#}$	25.00 ± 1.67$^{\#}$
Fluoxetine (20) + PTL (1)	143.63 ± 3.17*$^{\#}$	28.75 ± 2.31*$^{\#}$
Desipramine (20) + PTL (1)	166.13 ± 4.4$^{\#}$	19.75 ± 1.85$^{\#}$
Bupropion (20) + PTL (1)	178.50 ± 2.95$^{\#}$	17.88 ± 1.56$^{\#}$
Statistics	Interaction ($F(13,98) = 80.77$, $P < 0.05$)	Interaction ($F(13,98) = 29.98$, $P < 0.05$)

The values are expressed as mean ± s.e.m., n = 8 per group. The swimming episodes represent the number of quadrants crossed. *$P < 0.05$ compared with vehicle-treated group; $^{\#}P < 0.05$ compared with PTL-treated group.

Forced swim and tail suspension tests

The acute treatment with PTL (0.5–2 mg kg^{-1}) significantly increased the duration of immobility ($F(4,35) = 57.35$, $P < 0.05$) and decreased swimming episodes ($F(4,35) = 12.39$, $P < 0.05$) in mice compared with vehicle treatment (Figure 1B, C). This effect was dose dependent and was not observed at the lowest dose (0.25 mg kg^{-1}) tested. Likewise, a significant increase in duration of immobility ($F(4,35) = 11.17$, $P < 0.05$) was evident at the same dose levels in TST (Figure 1D).

Interaction study

There was an overall significant difference in duration of immobility ($F(13,98) = 80.77$, $P < 0.05$) and swimming episodes ($F(13,98) = 29.98$, $P < 0.05$) among the different treatment groups in the mouse FST interaction study. PTL (1 mg kg^{-1})-induced increased duration of immobility and decreased swimming episodes were reversed by all the antidepressants tested (Table 2).

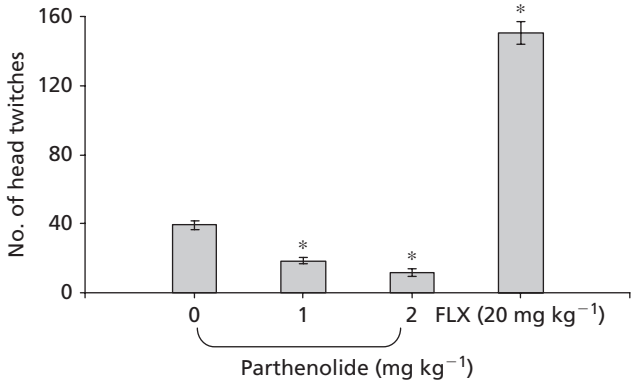

Figure 2 Effect of parthenolide and fluoxetine (FLX) on 5-hydroxytryptophan (5 mg kg^{-1}) + pargyline (75 mg kg^{-1})-induced head twitch response in mice. The columns represent mean number of head twitches and error bars indicate s.e.m., n = 8 per group. *$P < 0.05$ compared with vehicle-treated group (0 value on abscissa).

5-HTP-induced head twitch response

The combination of pargyline and 5-HTP induced the characteristic head twitch response (HTR). The antidepressant dose of fluoxetine (20 mg kg^{-1}) potentiated the HTR whereas pre-treatment with PTL (1 and 2 mg kg^{-1}) significantly ($F(3,28) = 44.12$, $P < 0.05$) attenuated the HTR (Figure 2).

Behaviour of OBX rats

The effect of PTL treatment on the behaviour of OBX/sham rats was analysed in three different situations as mentioned above (Table 3). In the modified open field exploration paradigm with OBX rats, chronic treatment with PTL (1 and 2 mg kg^{-1}) significantly increased the ambulation ($F(7,40) = 51.49$, $P < 0.05$) compared with vehicle treatment. Similarly, in the elevated plus maze exploration paradigm on OBX rats, PTL treatment significantly increased the percentage of total time spent in open arms ($F(7,40) = 46.64$, $P < 0.05$). On observing the social interaction behaviour, it was found that the time spent by OBX rats in active interaction was significantly reduced by PTL treatment ($F(7,40) = 30.91$, $P < 0.05$). The higher dose (2 mg kg^{-1}) of PTL was more effective and the baseline behaviour of sham-operated rats was not influenced by drug/vehicle treatments in all the above paradigms. Paroxetine (10 mg kg^{-1}) significantly decreased the bulbectomy-induced behavioural deficits in all the three situations.

Discussion

Parthenolide (PTL), in its pure form (at different dose levels), was evaluated in a battery of behavioural antidepressant assays in rodents. The forced swimming test (FST) takes advantage of the observation that rodents, following initial escape-oriented movements in an inescapable situation (in a cylinder filled with water), rapidly adopt a characteristic immobile posture (indicative of despair). It is also a useful tool in the better understanding of the role of specific monoamines and receptor subtypes implicated in depressive states (Redrobe & Bourin 1999). The tail suspension test (TST) is a similar and

Table 3 Effect of parthenolide on the behaviour of olfactory bulbectomized (OBX) rats in modified open field, elevated plus maze and social interaction paradigms

Treatment group (dose, mg kg^{-1})	Ambulation scores (modified open field)	Percentage of time spent in open arms (elevated plus maze)	Time spent in social interaction (s)
OBX + vehicle	165.00 ± 8.69	24.22 ± 1.05	90.27 ± 6.96
OBX + PTL (1)	222.67 ± 17.33*	44.72 ± 5.34*	55.83 ± 4.7*
OBX + PTL (2)	304.17 ± 15.81*	64.44 ± 5.81*	30.67 ± 2.3*
OBX + paroxetine (10)	83.33 ± 16.80*	15.67 ± 1.14*	135.55 ± 12.81*
Sham + vehicle	71.33 ± 6.24*	12.61 ± 0.91*	139.17 ± 10.6*
Sham + PTL (1)	68.67 ± 9.16	14.25 ± 2.86	146.17 ± 15.15
Sham + PTL (2)	73.21 ± 11.45	11.41 ± 1.12	141.88 ± 11.32
Sham + paroxetine (10)	76.17 ± 9.17	16.33 ± 3.24	148.17 ± 9.24
Statistics	$F(7,40) = 51.49$, $P < 0.05$	$F(7,40) = 46.64$, $P < 0.05$	$F(7,40) = 30.91$, $P < 0.05$

The values are expressed as mean ± s.e.m. n = 6 per group. The drug/vehicle treatments were carried out once a day for 14 days. *$P < 0.05$ compared with vehicle-treated OBX group.

simple behavioural model based on an immobility response to inescapable aversive stimulation (mice suspended by the tail). As in the FST, the immobility in the TST is sensitive (Steru et al 1985; Thierry et al 1986) to a wide variety of antidepressants. Interestingly, PTL showed depressant-like effects in both the FST and TST. The sedative effect of any test substance, leading to decline of performance in a behavioural task, is liable to be inferred as state of despair (Porsolt et al 1978; O'Neill & Moore 2003). However, PTL did not influence the locomotion of mice as observed from spontaneous locomotor activity (SLA) test. Moreover, the states of immobility in the FST and TST being situationally different (Steru et al 1985), it can be inferred that PTL induces a severe depressant-like effect that is not merely due to hypo-locomotion.

Though the depressant effects of PTL peaked at the highest dose level (2 mg kg^{-1}) tested, its almost equivalent (to peak effect) lower dose, 1 mg kg^{-1}, was selected for the interaction study. Antidepressants acting through different pharmacological mechanisms – venlafaxine (serotonin and noradrenaline (norepinephrine) reuptake inhibitor), escitalopram (highly potent and selective serotonin re-uptake inhibitor), citalopram and fluoxetine (selective serotonin re-uptake inhibitors), desipramine (tricyclic antidepressant), bupropion (noradrenaline and dopamine re-uptake inhibitor) – were administered as a pre-treatment to find out the resultant modulation of depressant-like effect of PTL. The antidepressants acting through the 5-HT mechanism (venlafaxine, escitalopram, citalopram, fluoxetine) were more effective in reversing the depressogenic effects of PTL, indicated by the significant difference in duration of immobility and swimming episodes of the interaction groups compared with that of vehicle treatment. Those antidepressants acting through other mechanisms (desipramine and bupropion), when given in combination with PTL, exhibited no significant difference in parameters when compared with the vehicle group.

Administration of 5-HTP, being the immediate precursor of 5-HT, was reported to increase the serotonergic transmission, inducing a characteristic head twitch (abrupt lateral movements) response in mice (Ortmann et al 1981; Schreiber et al 1995). Since 5-HTP is prone to enzymatic cleavage in blood, pre-treatment with a monoamine oxidase inhibitor, like pargyline, is essential (Nabeshima et al 1991). The reduction in the number of head twitches due to PTL pre-treatment indicates that, though 5-HT synthesis is augmented (by exogenous 5-HTP), its release from the pre-synaptic stores is inhibited by PTL, eventually leading to decreased serotonin neurotransmission.

OBX rats suffer a series of severe behavioural and neurochemical anomalies of which derangement in the serotonergic system and its behavioural consequences has been a special focus (Lumia et al 1992; Bourin et al 2001; Watanabe et al 2003; Mahesh et al 2007). The reduced re-uptake (Butler et al 1988), altered binding to imipramine and increase in number of $5\text{-}HT_{2A}$ receptors reported in the OBX rat simulate the condition as in patients suffering from depression (Briley et al 1980; Butler & Leonard 1988; Jesberg & Richardson 1988; Earley et al 1994). Though the exact neurochemical mechanisms underlying the behavioural deficits in bulbectomized rats are far from clear, this surgery-based model has been considered as a sensitive and chronic simulation of hyposerotonergic depression (Lumia et al 1992). The simple and predominantly used post-bulbectomy behavioural paradigm is the modified open field exploration in which OBX rats exhibit hyperactivity indicated by increased ambulation. Other prominent observations include open arm activity (generally increased in OBX rats) in the elevated plus maze (Ramamoorthy et al 2008) and decreased social interaction (Kelly et al 1997). From our study, it is clearly evident that chronic treatment with pure PTL potentiates the hyper-emotionality of OBX rats, as observed in all the three models.

The 5-HT uptake and release mechanisms in platelets (warehouse of 5-HT) and in serotonergic neurons were reported to be similar (Sneddon 1973) and there exists a strong liaison between platelet 5-HT and depression (Le Quan-Bui 1984; Camacho & Dimsdale 2000). The abnormalities in platelet signal transduction mechanisms involving protein kinase C (Morishita et al 1999), phosphatidylinositols (Soares et al 1999) and cyclic adenosine mono-phosphate (Perez et al 1999) have been linked to depression in man. It is plausible that the inhibitory effects of PTL on 5-HT release from platelets mediate depressant-like effect in rodents. Adhering to the orthodox monoamine hypothesis (Schildkraut 1965), PTL causes depressant-like effects due to 5-HT inhibitory effects in the CNS as observed from decreased swimming episodes in the FST, alleviation of 5-HTP-induced HTR and augmentation of OBX-induced behavioural deficits. Surprisingly, none of the clinical trials conducted thus far had reported depression or related symptoms occurring in patients treated with feverfew (Johnson et al 1985; Murphy et al 1988; Vogler et al 1998; Shrivastava et al 2006). The most convincing explanations might be that, firstly, this study has used the pure chemical form, hence a higher dose of PTL is being administered, compared with treatment with feverfew (in which PTL amounts to $\pm$ 1%) and, secondly, the presence of other bioactive principles in feverfew may neutralize the depressogenic effects of PTL.

Conclusions

This preliminary psychopharmacological investigation demonstrates that PTL treatment induces a depressant-like effect. Since the peripheral (platelet) and central (neuronal) mechanisms of 5-HT uptake and release are similar, it can be inferred that the pure chemical form of PTL (which is BBB permeable) might reduce the synaptic concentrations of 5-HT in the serotonergic neurons. Affinity studies on 5-HT transporters are essential to consider PTL-induced depression as a model to identify antidepressants acting through serotonergic mechanisms. The depressogenic property of PTL, demonstrated in rodents, has a notable implication on the clinical use of its (PTL) pure chemical form.

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