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5-Hydroxytryptamine-inhibiting property of Feverfew: role of parthenolide content

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KEY WORDS *Tanacetum parthenium*; Feverfew; parthenolide; sesquiterpenes; lactones; stomach fundus; ileum; serotonin antagonist; migraine

ABSTRACT

AIM: To study the mechanism of antimigraine activity of *Tanacetum parthenium* (Feverfew), its extracts and parthenolide, a component of Feverfew, by observing their effect on 5-HT storage and release, and stimulation of 5-HT_{2B} and 5-HT_{2A} receptors. Also to standardize a dosage form of Feverfew with respect to its parthenolide content. **METHODS**: Isometric responses to 5-HT and an indirect acting serotonergic, *d*-fenfluramine, were obtained on rat fundus and ileum. In one set of experiments the effect of dichloromethane extract of Feverfew and parthenolide was observed on the above. The extract was then thermally degraded upto 10 %, 23 %, and 33 % with respect to its parthenolide content by keeping at 60 ℃ and 75 % relative humidity and the experiments were repeated. In another set of experiments rats were fed with 20 mg/kg Feverfew powder (equivalent to a human dose of 500 μg parthenolide per day) for 30 d or were ip injected with parthenolide (23.4 μg/day) for 7 d. In the same set of experiments one group of rats were fed with 15 % and 77 % degraded Feverfew powder in the same dose as mentioned above. After 30 days the effects of the above were observed on 5-HT and *d*-fenfluramine. Feverfew was specially formulated and tested for stability under accelerated conditions. **RESULTS**: Parthenolide (1×10^{-5} mol/L) non-competitively antagonised the effects of *d*-fenfluramine but had no significant effect on 5-HT_{2B} and 5-HT_{2A} receptors in rat fundus and ileum at 30 min which turned significant on increasing the incubation time to 1.5 h, in rat fundus. Parthenolide (5×10^{-5} mol/L) followed the same trend. However, Feverfew extract (1×10^{-5} mol/L) potently and directly blocked 5-HT_{2B} and 5-HT_{2A} receptors and neuronally released 5-HT. At 5×10^{-5} mol/L the extract potently and irreversibly blocked the above. Both parthenolide and Feverfew extract showed a time-dependency in their action. The extract when degraded thermally upto 10 % could significantly block the 5-HT receptors and neuronal release of 5-HT, however, on further degradation it lost its inhibitory capacity markedly. Similar results were observed in rats fed orally with undegraded and degraded Feverfew powder and injected ip with parthenolide. Feverfew powder was more effective than any of its extracts or pure parthenolide. **CONCLUSION**: Feverfew powder is more potent than any of its extract or parthenolide alone in its antiserotonergic activity. Degraded Feverfew extracts show a marked decrease in their antiserotonergic activity. With thermally degraded Feverfew powder containing less contents of parthenolide no built-up antiserotonergic responses were observed after one month. This ascertains that Feverfew should be dispensed in a properly stabilized form wherein its parthenolide content is not degraded to less than 90 % of the original content.

INTRODUCTION

The herb Feverfew (*Tanacetum parthenium* L Shultz, Bip) is a traditional remedy for the prophylaxis of arthiritis, asthma and migraine headaches in Europe[1,2]. There has been a renewal of interest in the use of preparations from the Feverfew for their antimigraine activity for which fresh leaves gathered domestically or dried leaf preparations from health food shops are used. The antimigraine effect appears to be due to the presence of sesquiterpene butyrolactones present in the Feverfew leaves such as parthenolide which has shown an inhibitory

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action on serotonin release and platelet aggregation *in vitro*[3-8]. Evidence from platelet studies purported the idea that Feverfew and its active principle, parthenolide, exert an antimigraine action via a 5-hydroxytryptamine (5-HT) receptor based mechanism[6]. In a recent report parthenolide was shown to displace [^{3}H]ketanserine from 5-HT_{2A} receptors in rat and rabbit brain and cloned 5-HT_{2A} receptors suggesting that parthenolide may be a low affinity antagonist at 5-HT receptors[8].

Serotonin elicits contractions on rat stomach fundus and ileum mediated by stimulation of 5-HT_{2B} and 5-HT_{2A} receptors, respectively. The gastrointestinal tract has a high storage of 5-HT in mucosa and 5-HT neurons. Since activation of 5-HT_{2B} and 5-HT_{2A} receptors on rat stomach fundus and ileum can be monitored as the tension developed by this tissue, and since GIT contains abundant 5-HT stores in the intramural neurons[7], these preparations were used to study the interaction of 5-HT, *d*-fenfluramine, a specific 5-HT neuronal releaser, parthenolide and Feverfew at the receptor level.

The levels of parthenolide content in powdered leaf falls during storage[9]. Therapeutic efficacy, with respect to its anti-platelet aggregatory property and its 5-HT antagonising property falls with a decrease in the parthenolide content in Feverfew[9]. Degraded Feverfew extracts were tested to ascertain the level of degradation upto which the parthenolide showed an optimum inhibitory activity on 5-HT. Still, many stored Feverfew samples with negligible parthenolide also work therapeutically. Parthenolide and other active principles of Feverfew are known to form adducts with the sulfhydryl groups present in the body proteins[10-12]. So this study was designed to also see whether 5-HT-inhibiting activity of Feverfew powder with 85 % and 23 % parthenolide content builds up in the body tissues by sulfydryl (– SH) binding and attains therapeutic levels for antimigraine activity so as to be able to standardize a dosage form of Feverfew with respect to its parthenolide content.

MATERIALS AND METHODS

Methods Rat stomach fundus and ileum were obtained from 24-h fasted Wistar-Kyoto rats (200 – 250 g, either sex, from Central Animal Facility) and mounted in 10-mL organ chambers containing modified Krebs solution (in mmol/L: NaCl, 118.2; KCl, 4.6; $CaCl_2$, 1.6; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 24.8; glucose, 10). Resting tension of 1 g and 0.25 g was applied to rat fundus and ileum, respectively and tissues were equilibrated for a period of 1 h in physiological salt solution (PSS) at 37 ℃ oxygenated with carbogen. Contractility to 1×10^{-5} mol/L 5-HT was used as a reference control for maximum response and responses of 5-HT and *d*-fenfluramine were calculated as percentage of maximum response. Recordings were obtained on a "Gemini" two-channel recorder (UGO Basile, Italy) through an isometric transducer (UGO Basile, Italy). Solutions were prepared fresh before each experiment. Cumulative dose-response curves (CDRC) to 5-HT and *d*-fenfluramine were taken in rat fundus. In rat ileum experiments, fenpiverinium bromide (1×10^{-7} mol/L) was added to the PSS to minimize the spontaneous contractions of the cholinergic nature. Selegiline HCl (1×10^{-5} mol/L), prazosin (5×10^{-7} mol/L) and timolol (5×10^{-7} mol/L) were added to the PSS to prevent degradation of released 5-HT through the MAO enzymes and to prevent the influence of catecholamines in both rat fundus and ileum.

Experimental protocol

SET 1

In rat fundus, a CDRC of 5-HT was obtained. After repeated tissue washing, the fundal strips were incubated with a fixed concentration of parthenolide or a dichloromethane extract of Feverfew for either 30 min or 1.5 h. Responses to 5-HT were then obtained and a CDRC was plotted. Four different concentrations of parthenolide (1×10^{-7}, 1×10^{-6}, 1×10^{-5}, and 5×10^{-5} mol/L) were used out of which 1×10^{-5} mol/L and 5×10^{-5} mol/L were chosen to be represented in this study.

In the case of the 5-HT releaser, *d*-fenfluramine, the study was started with a CDRC of *d*-fenfluramine. Doses higher than 1×10^{-4} mol/L were not used as they are postulated to directly act on 5-HT_{2B} receptor sites[7]. After repeated washings, a fixed concentration of either parthenolide or dichloromethane extract was added for either 30 min or 1.5 h, and then a CDRC of *d*-fenfluramine was recorded again.

In case of degraded extracts, CDRC's of either 5-HT or *d*-fenfluramine were recorded. After washing, a fixed concentration of dichloromethane extract (10 %, 23 %, or 33 % degraded with respect to the parthenolide content) was added for either 30 min or 1.5 h and then a CDRC of either 5-HT or *d*-fenfluramine was recorded again.

SET 2

A group of rats were fed with Feverfew powder in a

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daily dose of 20 mg/kg containing 11.7 μg parthenolide (corresponding to a human dose of 500 μg of parthenolide per day)[9] or cyproheptadine (1.5 mg/kg) for 30 d prior to isolated tissue studies. A group was also ip injected with parthenolide (23.4 μg/day) for 7 d prior to isolated tissue studies. Feverfew powder was then degraded thermally upto 15 % and 75 % with respect to its parthenolide content and the degraded powder was fed to the rats for 30 d and isolated studies were then carried out. On day 30 of each group, the rat fundus was isolated after 24-h fasting and CDRC to 5-HT and *d*-fenfluramine were taken. The effect of Feverfew administration to rats for 30 d was also observed on histamine- and pilocarpine-mediated contractions in isolated rat fundus.

Drugs Serotonin creatinine sulfate, cyproheptadine hydrochloride, dextro-*d*-fenfluramine hydrochloride, prazosin hydrochloride, timolol maleate, risperidone, histamine dihydrochloride, pilocarpine nitrate, and parthenolide were purchased from Sigma Chemical Co, St Louis, MO, USA. Selegiline hydrochloride (USP Reference Standard) was purchased from E Merck (India) Ltd. Fenpiverinium bromide was purchased from Servicore Lab Pvt Ltd, India. All other chemicals were of AR grade.

All the drugs except parthenolide were soluble in water. Parthenolide (24.83 mg) was dissolved in Me_2SO (5 mL, final bath concentration less than 0.05 %) and stock solution of 100 mL was made with deionised water. Dry Feverfew powder (50 g) was extracted with dichloromethane exhaustively and the pooled extract was concentrated and dried *in vacuo*. The parthenolide content of the resultant dry extract was analyzed by HPLC and an amount of extract containing 24.83 mg was dissolved in 5 mL of Me_2SO and a 100-mL stock solution was made with deionised water. The stock solution was sonicated for 5 min and filtered through a 0.45-μm filter. Feverfew extract (5 g) was kept in an oven maintained at 60 ℃. Samples (500 mg) were withdrawn after 5, 10, and 15 d to get samples of various degrees of degradation. The samples were analyzed for parthenolide contents by HPLC to ascertain the extent of degradation. Feverfew powder was kept in an oven maintained at 60 ℃, samples were withdrawn after 7 and 15 days to get samples of various degrees of degradation. For feeding experiments, dry Feverfew powder containing 0.3 % parthenolide (400 mg) was suspended in 0.5 % xantham gum solution (50 mL) by ultra sonication. Degraded Feverfew powder samples were prepared as above using the same amount as in undegraded Feverfew powder. Fresh stocks of parthenolide, Feverfew extracts, and Feverfew powder were made after every 3 days and stored at 4 ℃.

Statistical analysis The tension developed to 5-HT and *d*-fenfluramine was either measured in grams and graphs were plotted against − log dose or responses to 5-HT, *d*-fenfluramine, histamine, and pilocarpine were calculated as percentages to the maximum response developed to 1×10^{-5} mol/L 5-HT in rat fundus. Paired and unpaired *t*-tests were used to evaluate the data and $P < 0.05$ was regarded as significant. Data are expressed as $\bar{x} \pm s_{\bar{x}}$.

RESULTS

Effect of pretreatment of isolated rat fundus and ileum with parthenolide and undegraded and degraded Feverfew extracts Parthenolide (1×10^{-5} mol/L) had no inhibitory effect on 5-HT_{2B} and 5-HT_{2A} receptors when the tissues were pretreated for 30 min (Fig 1, 2A). On increasing the incubation time to 1.5 h parthenolide significantly blocked the 5-HT_{2B} receptors in rat fundus (Fig 1). At a higher concentration of 5×10^{-5} mol/L, parthenolide followed the same trend in rat fundus (Fig 1).

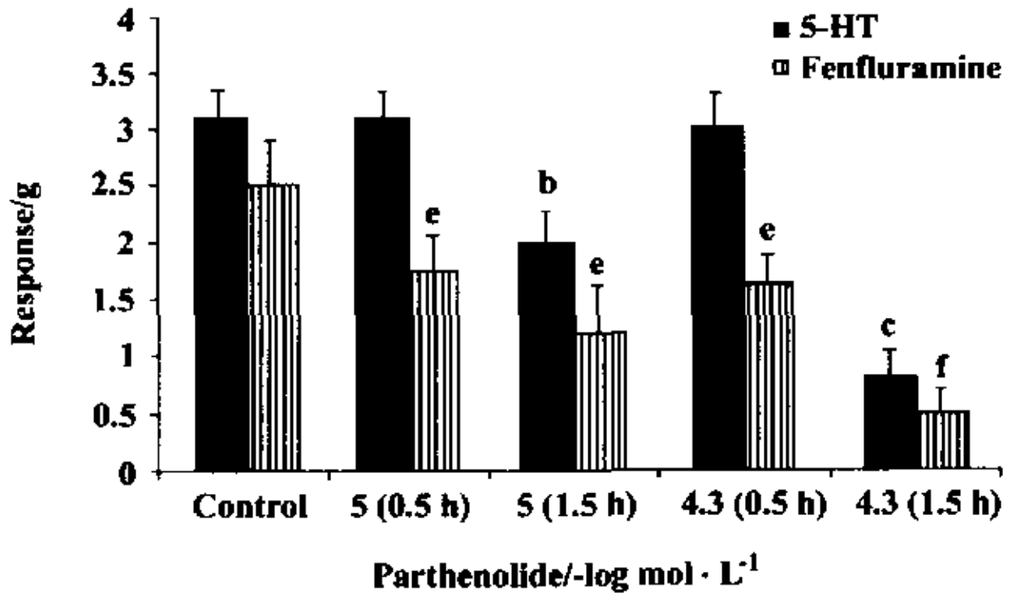

Fig 1. Effect of parthenolide on the 5-HT (10^{-5} mol/L)- and *d*-fenfluramine (10^{-4} mol/L)-mediated contractile responses in isolated rat fundus after 30 min and 1.5 h. $n = 10$ rats. $\bar{x} \pm s_{\bar{x}}$. $^bP < 0.05$, $^cP < 0.01$ *vs* 5-HT control. $^eP < 0.05$, $^fP < 0.01$ *vs* *d*-fenfluramine control.

However, *d*-fenfluramine-mediated responses were markedly inhibited by parthenolide (1×10^{-5} mol/L and 5×10^{-5} mol/L) at both 30 min and 1.5 h in rat fundus (Fig 1) and by 1×10^{-5} mol/L parthenolide in rat ileum

(Fig 2B).

Feverfew extract dose- and time-dependently inhibited the contractile responses to 5-HT (Fig 3A, 3B) and *d*-fenfluramine (Fig 4A, 4B). A marked inhibition of 5-HT_{2A} receptors and *d*-fenfluramine-mediated contraction was noted with 1×10^{-5} mol/L extract in rat ileum (Fig 2A, 2B).

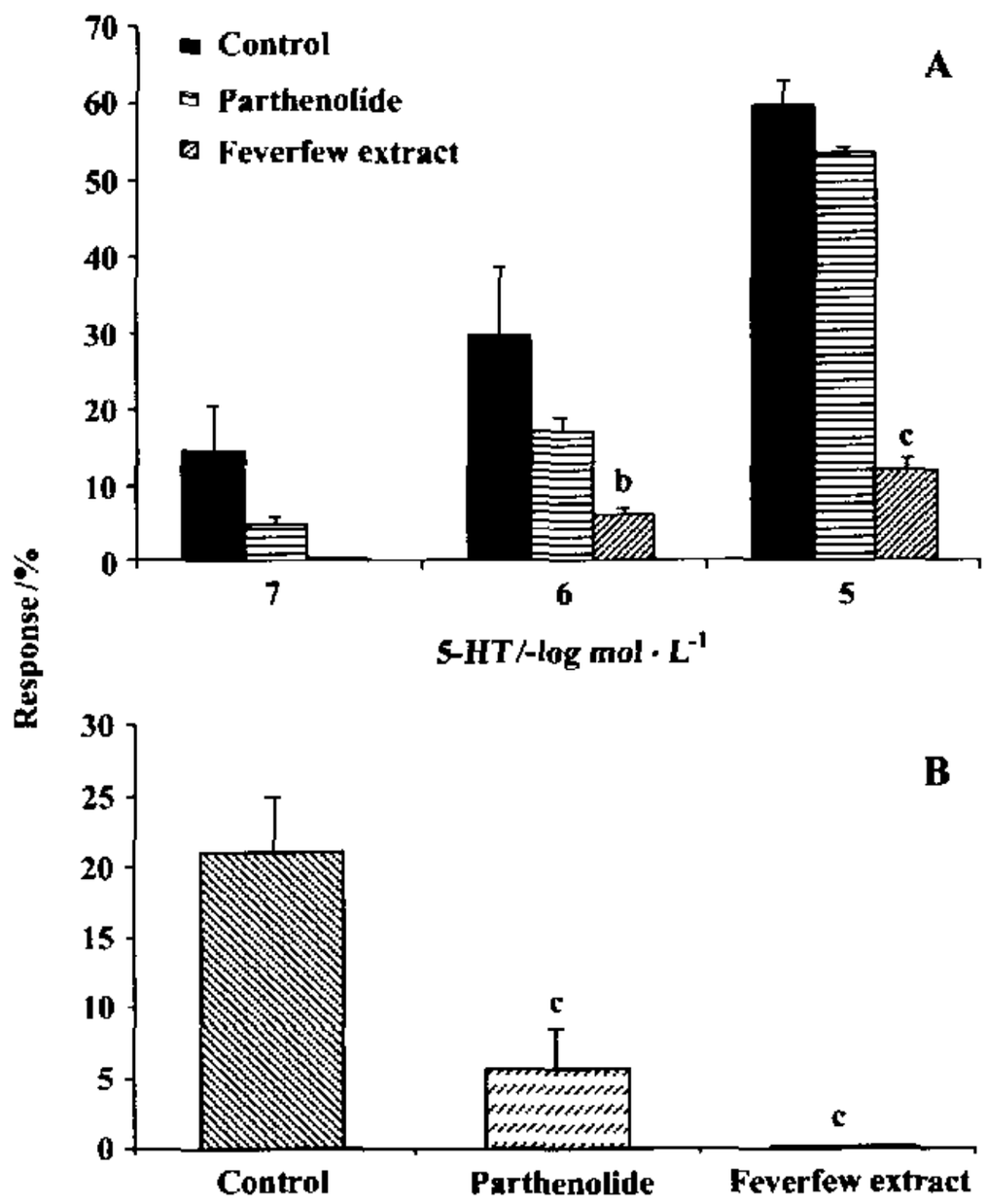

Fig 2. Effect of parthenolide (10^{-5} mol/L) and Feverfew extract containing equivalent concentration of parthenolide on spasmogenic responses in isolated rat ileum after 30 min. (A) Effect on 5-HT-mediated response. (B) Effect on *d*-fenfluramine (10^{-4} mol/L)-mediated response. % response was calculated taking the response of 10^{-5} mol/L 5-HT in rat fundus to be the maximum. $n = 10$ rats. $\bar{x} \pm s_{\bar{x}}$. $^bP < 0.05$, $^cP < 0.01$ *vs* control.

Degraded Feverfew extracts (10 %, 23 %, and 33 % degraded) showed a decrease in their inhibiting capacity on 5-HT_{2B}- and *d*-fenfluramine-mediated contractile responses (Fig 3C, 3D, 4C, 4D).

The contractile responses to 5-HT and *d*-fenfluramine were completely inhibited by cyproheptadine (5×10^{-5} mol/L) and risperidone (5×10^{-7} mol/L) in rat fundus and ileum respectively (data not shown).

Effect of *po* 20 mg/kg undegraded and degraded Feverfew powder Feverfew powder at a daily dose of 20 mg/kg containing 11.7 μg parthenolide (equivalent to 500 μg daily human dose) fed orally to rats for 30 d, significantly inhibited contractile responses to both exogenous 5-HT and neuronal release of 5-HT mediated by *d*-fenfluramine in isolated rat fundus (Fig 5A, 5B).

Degraded powder showed a considerable loss in its inhibitory activity on both exogenous 5-HT and neuronal release of 5-HT by *d*-fenfluramine in rat fundus (Fig 6A, 6B).

Cyproheptadine after a months feeding did not show any significant difference from the control results (Fig 5A, 5B, 6A, 6B). Parthenolide ip for 7 d significantly inhibited the *d*-fenfluramine-mediated neuronal release of 5-HT but not the exogenous 5-HT responses in rat fundus (Fig 7). Orally fed Feverfew powder was more effective than its alcoholic or dichloromethane extracts or parthenolide in its activity on 5-HT- and *d*-fenfluramine-mediated responses on rat fundus (Fig 7).

Undegraded Feverfew fed for 30 d also markedly inhibited the contractile responses to histamine and pilocarpine in rat fundus (Fig 8). Degraded powder showed a decrease in their antihistaminic and anticholinergic activity (Fig 8).

DISCUSSION

Feverfew has been widely used as a herbal remedy, especially for the prophylactic treatment of migraine. Although migraine is a complex neurovascular disorder, serotonin based mechanisms are central to its pathophysiology. Antimigraine drugs interact predominantly with receptors of 5-HT_1 and 5-HT_2 classes. 5-HT_{2B} and 5-HT_{2A} receptor antagonists such as methysergide, cyproheptadine, and mianserin have been shown to be effective in migraine prophylaxis. Effectiveness of Feverfew in migraine prophylaxis has been demonstrated in several clinical trials[13,15].

In the present study, parthenolide at an optimum concentration of 1×10^{-5} mol/L was observed to be a potent inhibitor of neuronal release of 5-HT, but without any significant direct effect on 5-HT_{2B} and 5-HT_{2A} receptor sites in rat fundus and ileum when the tissues were incubated for 30 min. Increasing the incubation time to 1.5 h resulted in a potent inhibition of both responses to exogenous 5-HT and neuronal release of 5-HT via *d*-fenfluramine. At a higher concentration (5×10^{-5} mol/L) parthenolide followed a similar trend as with 30-min

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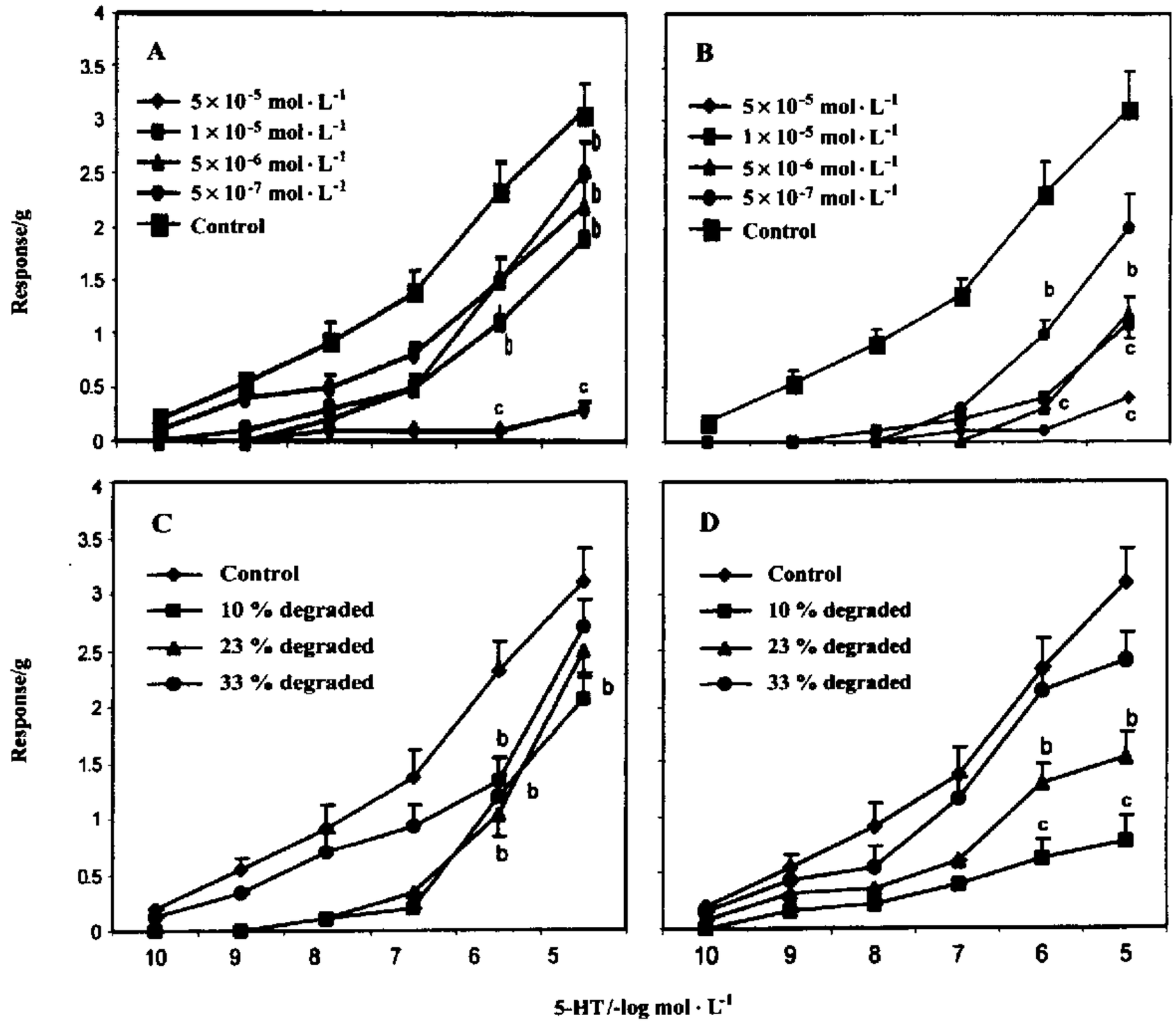

Fig 3. Effect of Feverfew extract on the spasmogenic response of 5-HT in isolated rat fundus. Effect of various concentrations of the extract after 30 min of incubation (A); after 1.5 h of incubation (B); Effect of different degrees of degradation of the extract after 30 min of incubation (C); after 1.5 h of incubation (D). $n = 5$ rats. $\bar{x} \pm s_{\bar{x}}$. $^bP < 0.05$, $^cP < 0.01$ *vs* control.

incubation but its antiserotonergic effect was much more striking when a 1.5-h incubation period was provided. The above results indicate that the antagonism at the 5-HT receptor sites is very slow. Cyproheptadine is reported to produce a slow antagonism in rat fundus taking about an hour to show a significant effect[14]. In a report by Bejar[7] no 5-HT_{2B} blocking action was noted with parthenolide (1×10^{-5} mol/L) whereas a significant inhibition of neuronally released 5-HT was seen. This may be due to a less incubation time (30 min) provided to parthenolide to act. Feverfew extracts containing 1×10^{-5} mol/L parthenolide and a number of other mono- and sesquiterpenes showed a potent inhibition of neuronally released 5-HT via *d*-fenfluramine and also 5-HT_{2B} and 5-HT_{2A} receptors in a manner similar to cyproheptadine (a predominantly 5-HT_{2B} receptor blocker) and risperidone (a 5-$HT_{2A/2C}$ receptor blocker) in rat fundus and ileum incubated with the extract for 30 min, respectively. If the incubation time was increased to 1.5 h or if a higher concentration of the extract was used (5×10^{-5} mol/L) an irreversible inhibition of serotonergic and of cholinergic and histaminergic responses were noted (data not shown). Thus it is evident that the plant extract which contains several other sesqui- and monoterpenes is more potent than parthenolide alone in its antiserotonergic activity. Plant powder, when fed for 30 days was even more effective than any extract of Feverfew.

Since parthenolide seems to be playing a major role in the antimigraine action of Feverfew, it has been suggested that manufacturers of Feverfew products should

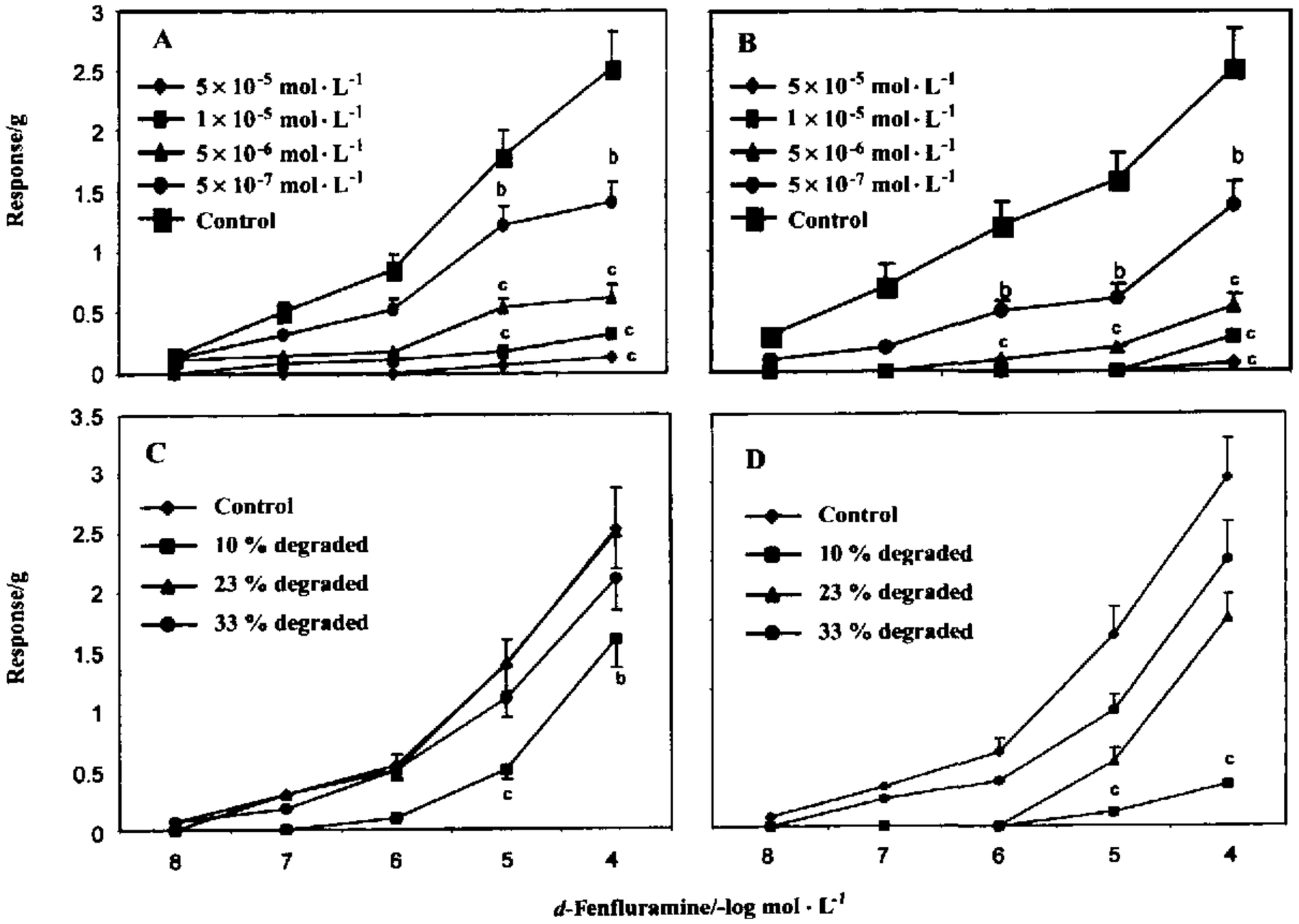

Fig 4. Effect of Feverfew extract on the *d*-fenfluramine-mediated spasmogenic responses in isolated rat fundus. Effect of various concentrations of the extract after 30 min of incubation (A); after 1.5 h of incubation (B). Effect of different degrees of degradation of the extract after 30 min of incubation (C); after 1.5 h of incubation (D). $n=5$. $\bar{x} \pm s_{\bar{x}}$. $^bP<0.05$, $^cP<0.01$ *vs* control.

keep parthenolide content as a means of standardization and quality control[9]. The level of parthenolide in powdered leaf material is reported to fall down considerably during storage[9]. Since parthenolide content is susceptible to degradation over long storage, Feverfew was specially formulated and tested for stability under accelerated conditions in our laboratory. To ascertain the activity on storage over a long period, the dichloromethane extract of Feverfew was thermally degraded upto 10 %, 23 %, and 33 % with respect to its parthenolide content by keeping it at 60 ℃ and 75 % relative humidity for varying number of days. The degraded extract could significantly block the 5-HT receptors and the neuronal release of 5-HT upto 10 % degradation with respect to the parthenolide content, however, any further degradation markedly reduced its 5-HT-inhibiting property. Hence it can be proposed that Feverfew in any form of powder or extract should be dispensed in a properly stabilized form wherein its parthenolide content is not degraded to less than 90 % of the original content.

Surprisingly, traditionally dried (slow drying in the shade) leaf powder has been shown to be still effective in migraine even though it may possess less parthenolide content[11,15]. Many commercial Feverfew preparations found to contain little or no parthenolide levels as analyzed by HPLC are still regarded as being effective against migraine by users[10]. Sequential treatment of old samples of dried powdered Feverfew leaf material, which contained no "free" parthenolide, with an oxidant (to convert the putative sulfide into the corresponding sulfone) and a weak base caused the regeneration of substantial amounts of parthenolide[10]. Parthenolide and other active principles of Feverfew are known to form adducts with the sulfhydryl groups present in the body proteins[11,12,14]. The sesquiterpene lactones contain an α-methylenebutyrolactone unit which is a potent Michael acceptor of the sulfhydryl groups[11]. This reaction is responsible for the inhibitory effect of Feverfew on aggregation of platelets[12]. The addition of cysteine and 2-mercaptoethanol to Feverfew extracts or to pure parthenolide

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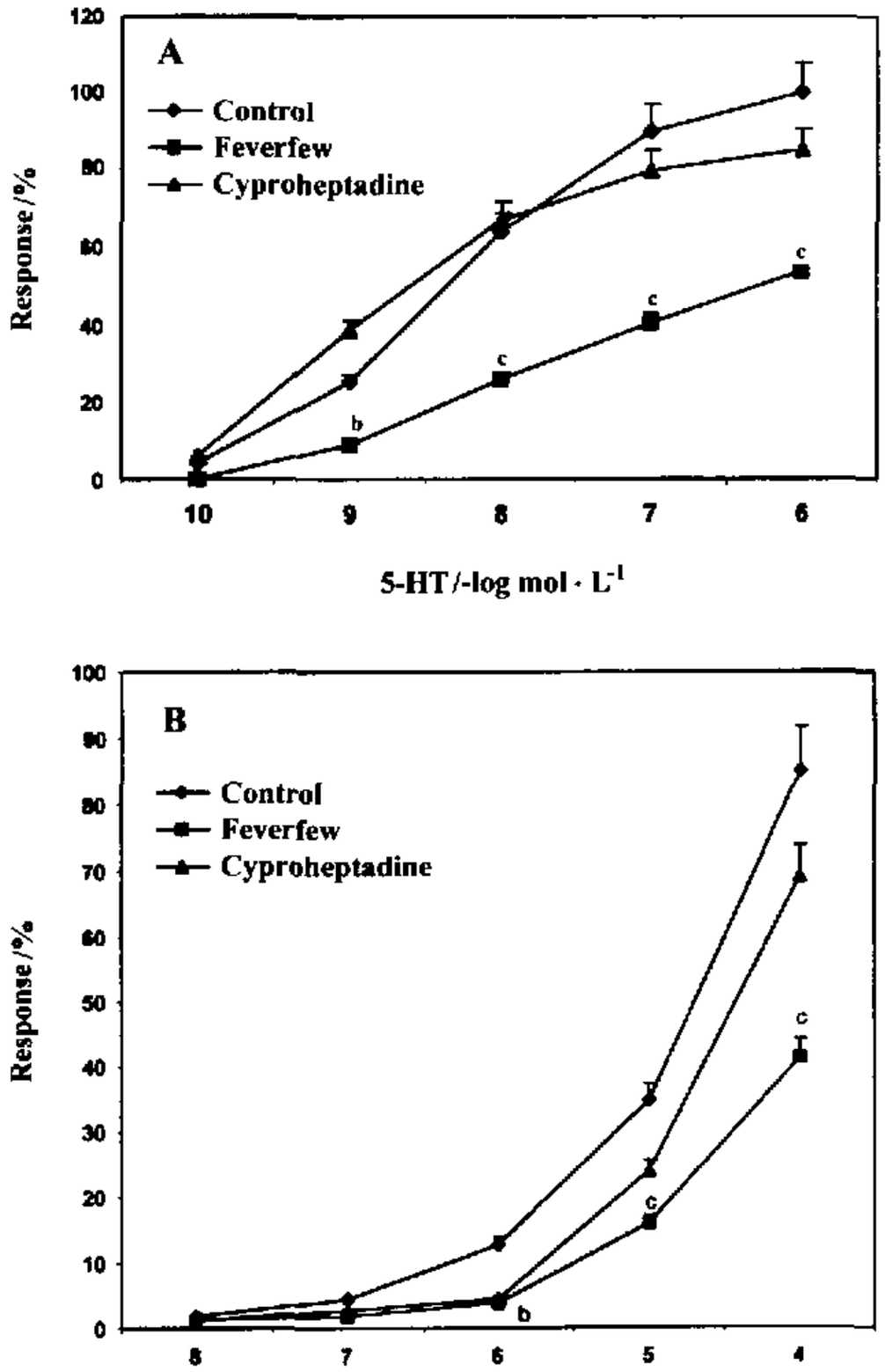

Fig 5. Effect of Feverfew (20 mg/kg, $n = 7$) and cyproheptadine (1.5 mg/kg, $n = 5$) fed to rats for 30 d on the spasmogenic response in isolated fundus. (A) Effect on 5-HT-mediated spasmogenic response. (B) Effect on *d*-fenfluramine-mediated spasmogenic response. $\bar{x} \pm s_{\bar{x}}$. $^bP < 0.05$, $^cP < 0.01$ *vs* control ($n =$ 10 rats).

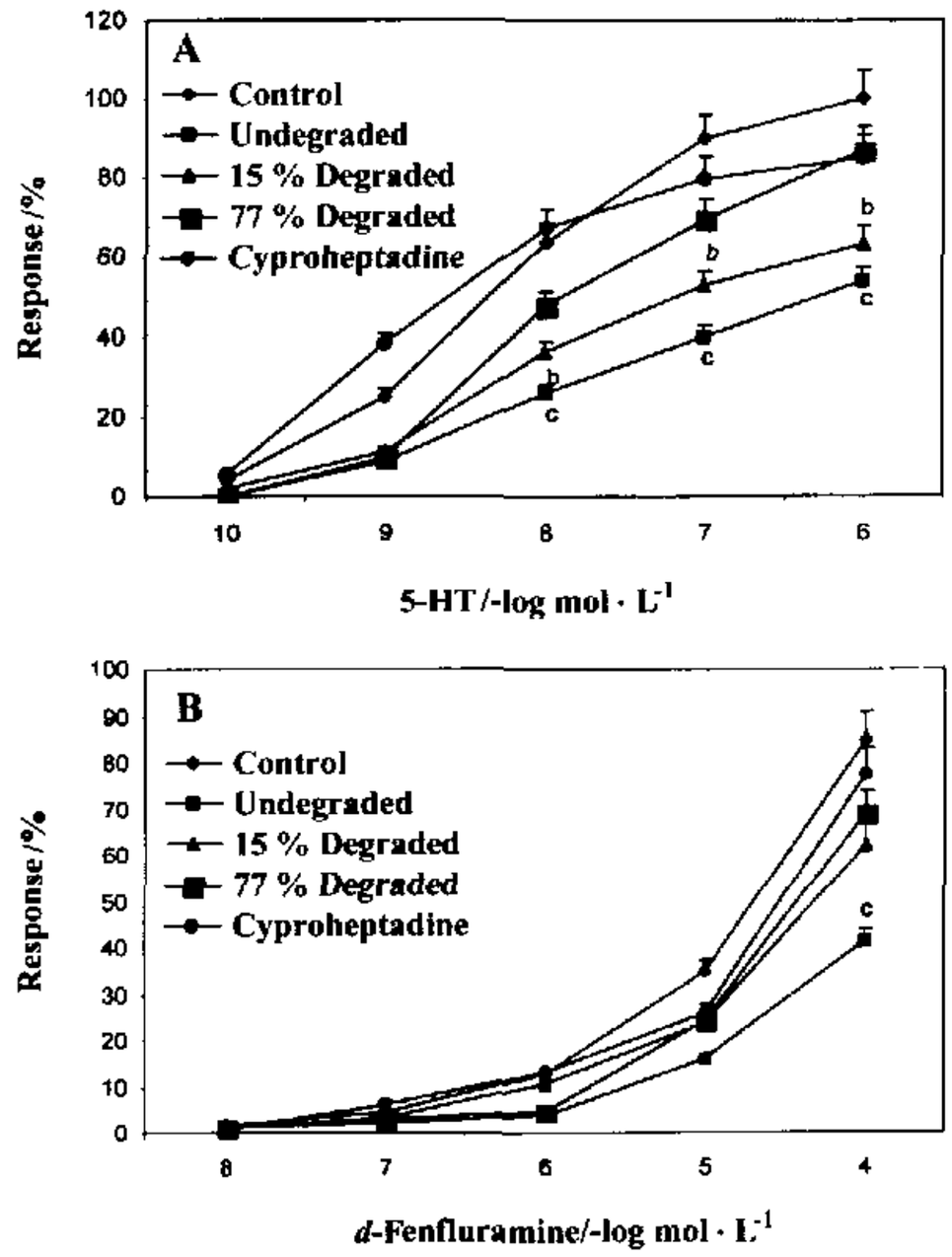

Fig 6. Effect of Feverfew (20 mg/kg) at different degrees of degradation (undegraded, $n = 7$; 15 % degraded, $n = 8$; 77 % degraded, $n = 6$) and cyproheptadine (1.5 mg/kg, $n = 5$) fed to rats for 30 d on the spasmogenic response in isolated fundus. (A) Effect on 5-HT-mediated spasmogenic response. (B) Effect on *d*-fenfluramine-mediated spasmogenic response. $\bar{x} \pm s_{\bar{x}}$. $^bP < 0.05$, $^cP < 0.01$ *vs* control ($n = 10$).

was seen to completely suppress their ability to inhibit platelet aggregation[12] and these inhibitory effects were also observed to be dose- and time- dependent[12]. Thus it may be possible that low amounts of parthenolide administered over long periods form adducts with the –SH groups of body proteins. And *in vivo*, glutathione or other thiol Michael adducts could be converted back into "free" α-methylenebutyrolactones on oxidation with cytochrome P-450 enzymes. To test this hypothesis rats were fed with 20 mg/kg Feverfew powder (equivalent to a daily human dose of 500 μg)[9,15] for 30 d or were injected ip with parthenolide for 7 d. The dose- and time-dependency of the anti-serotonergic effect of parthenolide

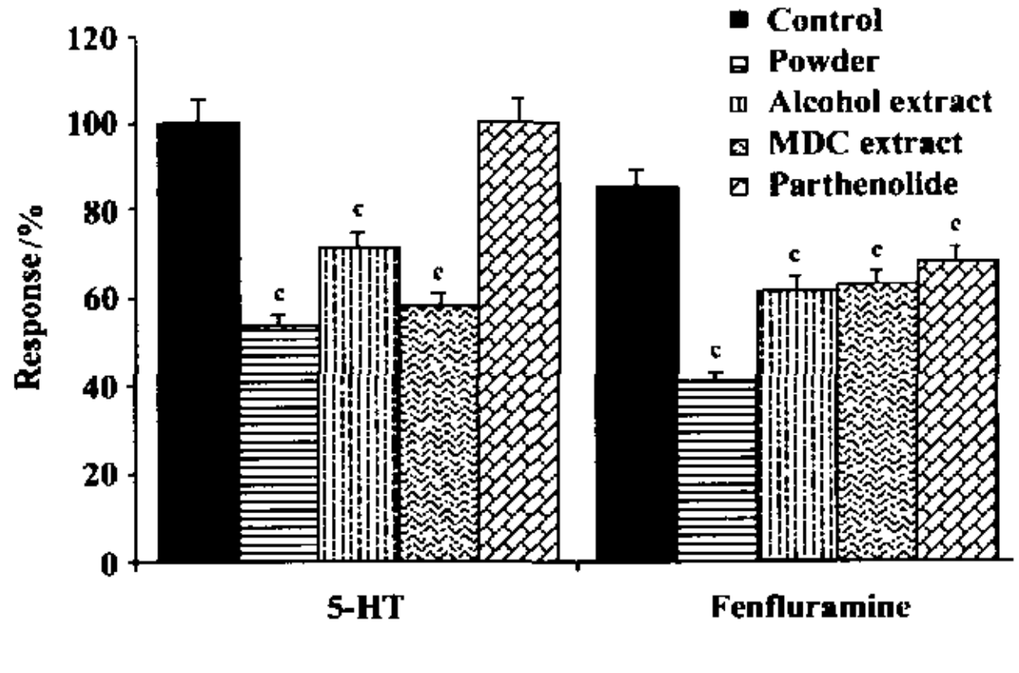

Fig 7. Effect of parthenolide (ip 0.06 mg/kg, $n = 5$), different extracts of Feverfew [equivalent to 0.06 mg/kg parthenolide added directly in the organ bath; alcoholic extract, $n = 4$; dichloromethane extract (MDC), $n = 8$], and powdered Feverfew (*po* 20 mg/kg for 30 d, $n = 7$) on 5-HT- and *d*-fenfluramine-mediated spasmogenic responses in isolated rat fundus. $\bar{x} \pm s_{\bar{x}}$. $^cP < 0.01$ *vs* control ($n = 10$).

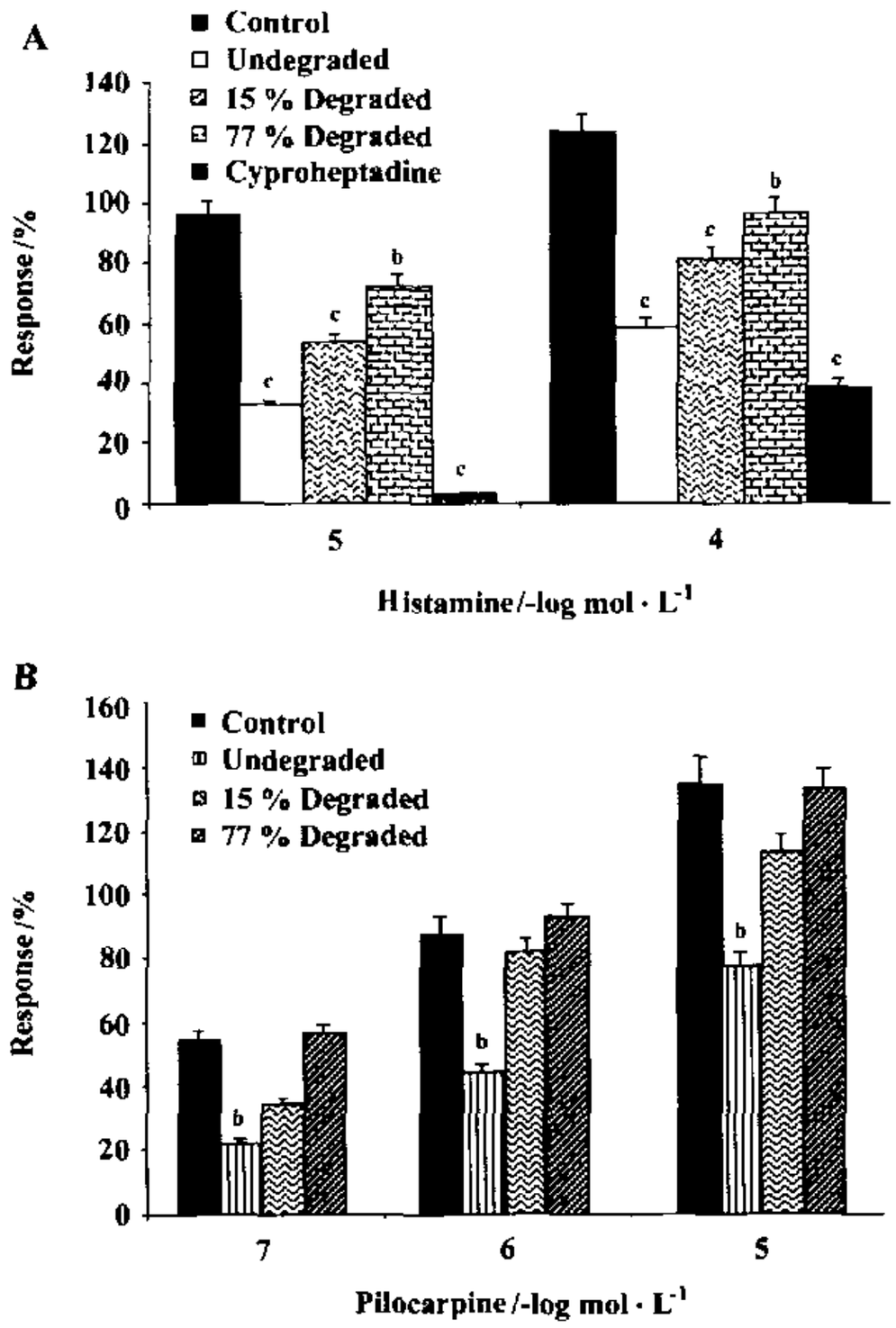

Fig 8. Effect of Feverfew (20 mg/kg) at different degrees of degradation (undegraded, $n=7$; 15 % degraded, $n=8$; 77 % degraded, $n=6$) fed to rats for 30 d on the spasmogenic response in isolated fundus. (A) Effect on histamine-mediated spasmogenic response with respect to cyproheptadine (*po* 1.5 mg/kg, $n=5$). (B) Effect on pilocarpine-mediated spasmogenic response. % response of histamine and pilocarpine is with respect to 10^{-5} mol/L 5-HT. $\bar{x} \pm s_{\bar{x}}$. $^bP<0.05$, $^cP<0.01$ *vs* control ($n=10$).

and Feverfew extract in our isolated tissue study made this an attractive hypothesis. However, this proposition did not hold true in our studies as when fed with lower quantities of parthenolide, as in 15 % and 77 % degraded powder, for one month, the isolated rat fundus did not show any built up inhibition of 5-HT responses. In fact, the anti-serotonin activity remained till the parthenolide content was 85 % of the original, below that there was a complete loss observed at 33 % parthenolide content. This leads to the contention that on traditional drying parthenolide in Feverfew may be forming adducts with thiols or other nucleophilic centres in plant intrinsic proteins and on ingestion the adduct regenerates parthenolide to exert its effect. That the parthenolide reacts with certain proteins and forms an adduct which gets regenerated with oxidants was evidenced in preliminary studies in our lab wherein the parthenolide peak in a reverse phase HPLC disappeared on addition of cysteine and reappeared on oxidation in an alkaline medium (unpublished observations). Hence the traditionally dried Feverfew leaf powder may still be useful in migraine provided its parthenolide content (free or complexed) is not below 90 %. The vigrous thermal degradation in our case must have broken down the parthenolide structure which could no longer form an adduct. As Feverfew powder is difficult to standardize because of the unstable nature of parthenolide, a Feverfew formulation may be preferable wherein the parthenolide content does not fall below 90 %. Degraded extracts also showed considerable loss of antihistaminic and anticholinergic activities.

Thus it was ascertained that the parthenolide content cannot be allowed to be degraded to less than 90 % of the original content. The Feverfew formulation developed in our laboratory was found to be more stable than Feverfew powder alone. It was also found to be more stable than other commercial preparations available, having a shelf life of 2 years with the parthenolide content not falling below 90 % by this period. Also, that the antihistaminic activity noted in our study may be attributing to Feverfew's antiallergic property and the anticholinergic activity to its analgesic property[16] in addition to its antiserotonergic property, all of which together may be useful in migraine prophylaxis.

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