Anticonvulsant effects of aerial parts of *Passiflora incarnata* extract in mice: involvement of benzodiazepine and opioid receptors
Marjan Nassiri-Asl*1, Schwann Shariati-Rad2 and Farzaneh Zamansoltani3

Address: 1Department of Pharmacology, School of Medical Sciences, Qazvin University, Qazvin, Iran, 2School of Medical Sciences, Qazvin University, Qazvin, Iran and 3Department of Anatomy, School of Medical Sciences, Qazvin University, Qazvin, Iran

Email: Marjan Nassiri-Asl* - marjan_nassiriaslm@yahoo.com; Schwann Shariati-Rad - dschwann@yahoo.com; Farzaneh Zamansoltani - zamansoltani@gmail.com

* Corresponding author

Abstract

**Background:** Passion flower (*Passiflora incarnata*) is used in traditional medicine of Europe and South America to treat anxiety, insomnia and seizure. Recently, it has shown antianxiety and sedative effects in human.

**Methods:** In this study, anticonvulsant effects of hydro-alcoholic extract of *Passiflora*, Pasipay, were examined by using pentylentetrazole model (PTZ) on mice. Pasipay, diazepam, and normal saline were injected intraperitoneally at the doses 0.4–0.05 mg/kg, 0.5–1 mg/kg and 10 ml/kg respectively 30 minutes before PTZ (90 mg/kg, i.p). The time taken before the onset of clonic convulsions, the duration of colonic convulsions, and the percentage of seizure and mortality protection were recorded. For investigating the mechanism of Pasipay, flumazenil (2 mg/kg, i.p) and naloxone (5 mg/kg, i.p) were also injected 5 minutes before Pasipay.

**Results:** An ED50 value of Pasipay in the PTZ model was 0.23 mg/kg (%95 CL: 0.156, 0.342). Pasipay at the dose of 0.4 mg/kg prolonged the onset time of seizure and decreased the duration of seizures compared to saline group (p < 0.001). At the dose of 0.4 mg/kg, seizure and mortality protection percent were 100%. Flumazenil and naloxone could suppress anticonvulsant effects of Pasipay.

**Conclusion:** It seems that Pasipay could be useful for treatment absence seizure and these effects may be related to effect of it on GABAergic and opioid systems. More studies are needed in order to investigate its exact mechanism.

Background

Epilepsy is one of the most common serious neurological conditions. In contemporary society, the frequency and importance of epilepsy can hardly be overstated from the epidemiologic studies. However, in most studies, the overall incidence of epilepsy in developed societies has been found to be around 50 cases per 100,000 persons per year, and rises steeply in older age [1,2]. The current therapeutic treatment of epilepsy with modern antiepileptic drugs (AEDs) is associated with side-effects, dose-related and chronic toxicity, and teratogenic effects, and approximately 30% of the patients continue to have seizures with current AEDs therapy [1,3].

Natural products from folk remedies have contributed significantly in the discovery of modern drugs and can be an alternative source for the discovery of AEDs with novel structures and better safety and efficacy profiles [4]. Now,
In Iran, several herbs have been used for anticonvulsant effects. The possibility of revival of traditional treatments on the basis of evidence-based medicine has raised the evaluation of Medieval Iranian medical remedies. In recent years, some experimental studies have evaluated the anticonvulsant effects of natural products or derivatives of natural products. Half of the medically important pharmaceutical drugs are derived from natural products. Herbal medicines are often considered to be a gentle and safe alternative to synthetic drugs. More than 40% of the population in the industrialized world which use herbal medicines as a supplement or substitute for prescription drugs are increased. Herbal medicines are considered to be a gentle and safe alternative to synthetic drugs. More than half of the medically important pharmaceutical drugs are either natural products or derivatives of natural products. In a recent study, the anticonvulsant effects of a natural product were evaluated in a PTZ model, which may be due to several mechanisms underlying the actions of Pasipay on the CNS and assessed the probable involvement of GABAergic and opioid system.

Methods

Animal

Male BALB/c mice (25–30 g) were obtained from the Razi Institute (Karaj, Iran). The animals were individually housed in colony rooms with 12/12 h light/dark cycle at 21 ± 2°C and had free access to food and water. All animal experiments were carried out in accordance with the regulations of the Ethics Committee of the Qazvin University of Medical Sciences.

Plant material

Hydro-alcoholic extract of Pasipay was obtained from Iran Darouk Pharmaceutical Co. (Tehran, Iran) which was prepared from the standardized extract of leaves, flower and fruit of P. incarnata. The total flavonoid content in hydro-alcoholic extracts related to the dried plant material was 4% (w/w) including vitexin and rutin.

Chemicals

Drugs used as follows: PIZ (Sigma), flumazenil ampoule (2 mg/kg) (Roche), diazepam (Chemidar, Iran), naloxone (Tolid Daru, Iran). PIZ, diazepam and naloxone were dissolved in normal saline. All compounds were prepared freshly each time and administered intraperitoneally.

Anticonvulsant activity

PTZ-induced seizure

The mice were divided into groups of ten animals each. In the four groups, the mice were given Pasipay at the doses (0.05, 0.1, 0.2, 0.4 mg/kg i.p.) 30 min before the administration of PIZ (90 mg/kg i.p.). Two groups were injected diazepam (0.5, 1 mg/kg i.p.) and one group was injected normal saline 30 min before the administration of PIZ (90 mg/kg i.p.) [23]. Each animal is placed into an individual plastic cage for observation lasting 1 h. The onset of a general clonus was used as the endpoint. The general clonus was characterized by forelimb clonus followed by full clonus of the body. The time taken before the onset of clonic convulsions, the duration of clonic convulsions, and the percentage of seizure and mortality protection were recorded [23].

The effect of flumazenil on the anticonvulsant activity of Pasipay

We also studied the effects of a selective benzodiazepine receptor antagonist, flumazenil on the anticonvulsant activity of Pasipay in order to investigate the probable involvement of benzodiazepine receptors. It was selected six groups of ten mice each. In the first group, mice were given flumazenil (2 mg/kg) 5 min before the administration of Pasipay (0.4 mg/kg) and 35 min before the administration of PIZ (90 mg/kg i.p.).
the injection of PTZ. In the second group, the animals received flumazenil (2 mg/kg) 5 min before the administration of diazepam (0.5 mg/kg). Also, three groups were injected diazepam (0.5 mg/kg i.p.), flumazenil (2 mg/kg) and normal saline 30 min before the administration of PTZ (90 mg/kg i.p.) respectively [23-26]. The anticonvulsant activity of Pasiyap and diazepam in mice pretreated with flumazenil was assessed and compared with normal saline (10 ml/kg), flumazenil (2 mg/kg), diazepam (0.5 mg/kg) and Pasiyap (0.4 mg/kg) treated animals.

The effect of flumazenil on the anticonvulsant activity of Pasiyap

It was selected four groups of ten mice each for further investigation the probable modulatory activities of opioid receptors on the anticonvulsant activity of Pasiyap [27,28]. It was applied naloxone as an opioid receptor antagonist at a dose of (5 mg/kg) 5 min before the administration of Pasiyap (0.4 mg/kg) and 35 min before the injection of PTZ in group of ten mice each [23-26]. The anticonvulsant activity of Pasiyap in groups pretreated with naloxone was assessed and compared with animals pretreated only with Pasiyap (0.4 mg/kg), naloxone (5 mg/kg) and normal saline (10 ml/kg) groups.

Statistical analysis

The dose of Pasiyap to produce an anticonvulsant (ED50) effect in 50 % of animals and its associated 95% confidence limits was calculated by Litchfield and Wilcoxon methods (PHARM/PCS Version 4). The data were expressed as mean values ± S.E.M. and tested with one-way ANOVA followed by the multiple comparison test of Tukey-Kramer. Results with p < 0.05 were taken significant.

Results

PTZ-induced seizure

An ED₅₀ value of Pasiyap in the PTZ model was 0.2 mg/kg (%95 CL: 0.156, 0.342). Pasiyap at the dose of 0.4 mg/kg prolonged the onset time of seizure and decreased the duration of seizures compared to saline group (p < 0.001) (Table 1). As it is shown in Table 1, Pasiyap exhibited its protection against seizure in a dose-dependent manner. Furthermore, diazepam prolonged the latency and shortened the duration of seizures compared to saline group (Table 1).

The effect of flumazenil on the anticonvulsant activity of Pasiyap

In the PTZ-induced seizure model, the administration of flumazenil (2 mg/kg) 5 min before Pasiyap (0.4 mg/kg) reversed the effect of Pasiyap in prolonging seizure latency and reducing the duration of clonic seizures. There was no significant difference between the latency and duration of seizure in mice which received Pasiyap (0.4 mg/kg) pretreated with flumazenil and the saline group. Also, flumazenil could reverse the anticonvulsant activity of diazepam (Table 2).

The effects of naloxone on the anticonvulsant activity of Pasiyap

Pretreatment of mice with naloxone (5 mg/kg) 5 min before the administration of the Pasiyap (0.4 mg/kg) reversed the reduction in seizure duration. However, the time course of the seizure threshold in mice was not reversed completely by naloxone and it was significant compared to control (p < 0.001) (Table 3).

Discussion

The present study investigated the anticonvulsant effect of Pasiyap using the PTZ-model. Pasiyap could suppress onset and duration of clonic seizure in PTZ model and it seems that this effect increased dose dependently. Also seizure and mortality protection percent increased dose dependently as we could observe that at the dose of 0.4 mg/kg, all animals were protected against seizure and mortality significantly and this effect was similar to diazepam 1 mg/kg.

This study is in agreement with a recent report by Dhawan et al, however, we have seen anticonvulsant effects of

Table 1: Effects of Pasiyap on PTZ-induced convulsion in mice

| Treatment (dose) | Onset (sec) | Duration (sec) | Seizure protection (%) | Mortality protection (%) |
|-----------------|-------------|----------------|------------------------|-------------------------|
| Normal saline (10 ml/kg) | 51.83 ± 64 | 12 ± 1.80 | 0 | 0 |
| Diazepam (0.5 mg/kg) | 485.5 ± 74.97*** | 3.5 ± 2.21* | 80 | 90 |
| Diazepam (1 mg/kg) | 600 ± 0*** | 0 ± 0*** | 100 | 100 |
| Pasiyap (0.05 mg/kg) | 56 ± 4.51 | 15.3 ± 4.48 | 0 | 0 |
| Pasiyap (0.1 mg/kg) | 112.66 ± 16.2 | 7.1 ± 2.5 | 10 | 50 |
| Pasiyap (0.2 mg/kg) | 137.6 ± 17.8*** | 7 ± 1.1 | 20 | 80 |
| Pasiyap (0.4 mg/kg) | 600 ± 0*** | 0 ± 0*** | 100 | 100 |

Normal saline, diazepam and Pasiyap were administered i.p. 30 min before the injection of PTZ (90 mg/kg, i.p.). Values are the mean ± S.E.M. for 10 mice. *p < 0.05, ***p < 0.001, compared to saline group, Tukey-Kramer test.
extract at the lower doses. This could be explained by several reasons: Our extract was the standard hydroalcoholic extract of aerial parts of herb which was prepared as the drug formulation, Pasipay, by Iran Darouk Pharmaceutical Co. But, the previous work was the methanolic extract of the leaves of *Passiflora incarnata* [19]. Moreover, there are several controversial reports about the CNS effects of *P. incarnata* extracts and their active component which could be related to different active component of it [29-33]. Meanwhile, in our study, the major flavonoids of Pasipay were also different from previous studies. In addition, there is a different between the sources of *P. incarnata* of our work and previous work.

Clonic seizure was induced by γ-aminobutyric acid (GABA) transmission blocker PTZ [34]. Regarding the possible contribution of GABAergic system in the anticonvulsant activity of Pasipay, flumazenil, a benzodiazepine receptor antagonist, was used [24]. As it was shown in table 2, flumazenil decreased the prolongation of seizure latency induced by Pasipay and it also antagonized the effect of Pasipay on decreasing the duration of clonic seizures in the PTZ model. It is noteworthy that the anticonvulsant effect of Pasipay is blocked by an antagonist of benzodiazepine receptor. So this effect of Pasipay seems to be related to benzodiazepine receptor activation. However, there is a controversial study which reported that pure vitexin and isovitexin of *P. incarnata* had no activity in CNS tests [35]. Further studies need to make clear which of these flavonoids or other compounds have anticonvulsant effects.

We also found other mechanism about the anticonvulsant effects of Pasipay. As it was shown in table 3, naloxone only antagonized the effect of Pasipay on decreasing the duration of clonic seizures in the PTZ model compared to saline group. Naloxone decreased the prolongation of seizure latency induced by Pasipay. However, it did not show any significant reversal of Pasipay effects. It seems that some part of anticonvulsant effects of it related to activation of opioid system which was attenuated by naloxone. Thus, we used naloxone as a non-specific opioid receptor antagonist for preliminary study to clear the exact mechanism of this herb.

On the other hand, anticonvulsant activity of kappa opioid receptor (KOPr) agonists has been established in wide range of previous animal studies. KOPr agonists are effective against bicuculline-, maximal electroshock- and excito-
tatory amino acid-induced convulsions. Furthermore, they attenuate the kindling of seizures produced by repeated administration of PTZ [36-39]. Furthermore, dynorphine, an endogenous opioid peptide, binds to KOPr. It has anticonvulsant effects in previous studies [28]. There is one hypothesis that Pasipay could activate KOPr and produce protective effects against PTZ-induced seizure. However, the mechanism of anticonvulsant effects with KOPr agonist, have not been universal. Modulatory effects of its agonist on seizure induced by GABA_A receptor antagonists were reported [36]. Furthermore, its agonist could inhibit glutamate release [40,41]. Thus, there are two possibilities which could explain the anti-convulsant activity of the Pasipay via the KOPr activation: 1) enhancement GABAergic activity or 2) attenuation glutamatergic activity.

Conclusion
In brief, the present study provides evidence for anticonvulsant activity of Pasipay in the clonic seizure of PTZ model. As the protective effects of Pasipay in clonic seizure it suggests that it could be useful for treatment of absence seizure. Furthermore, the important role of benzodiazepine receptor in the effects of Pasipay should be considered.

Also, the opioid receptor mechanism is apparently involved in the response induced by Pasipay which should be investigated.

Competing interests
The author(s) declare that they have no competing interests.

Authors’ contributions
MNA is the primary author and wrote the manuscript, participated in the design of the study and performed the statistical analysis and revised the manuscript. SSR and FZ helped in the design of the study and wrote the manuscript. All of the authors have read and approved the final manuscript.

Acknowledgements
The authors are thankful to Iran Darouk Pharmaceutical Co. (Tehran, Iran) for giving extract and certification analysis of *P. incarnata*.

References
1. Poole K, Moran N, Bell G, Solomon J, Kendall S, McCarthy M, McCormick D, Nashel L, Johnson A, Sander J, Shorrn Y: Patients’ perspectives on services for epilepsy: a survey of patient satisfaction, preferences and information provision in 2394 people with epilepsy. *Seizure* 2000, 9(8):551-558.
2. Ropper AH, Brown RH: *Epilepsy and other seizures disorder*. In Adams and Victor’s Principles of Neurology 8th edition. Edited by: Ropper AH, Brown RH. New York: McGraw-Hill; 2005:271-297.
3. Samren EB, van Duijn CM, Koch S, Hilemsa VA, Klepel H, Bardy AH, Manuageta GB, Deichi AW, Gally E, Granstrom ML, Meinardi H, Grobbe DE, Hofman A, Jn D, Lindhout D: Maternal use of antiepileptic drugs and the risk of major congenital malfor-

mations: a joint European prospective study of human tera-
togenesis associated with maternal epilepsy. *Epilepsia* 1997, 38(9):981-990.
4. Raza M, Shaheen F, Choudhury MI, Rahman AU, Sombasi S, Siria A, Rafiq A, Delorenzo RJ: Anticonvulsant effect of FS-1 subfrac-
tion isolated from roots of *Delphinium Denudatum* on hippoc-
amal pyramidal neurons. *Phytotherapy Res* 2003, 17(1):38-43.
5. Nsour WM, Lau CB, Wong SC: Review on psychotherapy in epi-lesy. *Seizure* 2000, 9(2):96-107.
6. Sucher NJ: Insights from molecular investigations of tradi-
tional Chinese herbal stroke medicines: Implications for neuroprotective epilepsy therapy. *Epilepsy Behav* 2006, 2(2):350-362.
7. Koehn FE, Carter GT: The evolving role of natural products in drug discovery. *Nat Rev Drug Discov* 2005, 4(3):206-220.
8. Newman DJ, Cragg GM, Snader KM: Natural products as sources of new drugs over the period 1981-2002. *J Nat Prod* 2003, 66(7):1022-1037.
9. Gorji A, Khaleghi Ghadiri M: History of epilepsy in Medieval Ira-nian medicine. *Neurosci Biobehav Rev* 2001, 25(5):455-461.
10. Zargari A: Medicinal plants Tehran: Tehran University; 1989.
11. Kinghorn GR: Passion, stigma, and STI. *Sex Transm Inf* 2001, 77(S):370-375.
12. Dhawan K, Dhawan S, Sharma A: Passiflora: a review update. *J Ethnopharmacol* 2004, 94(1):1-23.
13. Akhondzadeh S, Naghavi HR, Vazirian M, Shayeeganolou A, Rashidi H, Khani M: Passionflower in the treatment of generalized anx-iety disorder: a pilot double-blind randomized controlled trial with oxazepam. *J Clin Pharm Ther* 2001, 26(5):363-367.
14. Reginatto FH, De-Paris F, Petry RD, Quevedo J, Ortega GG, Gos-
man G, Schenkel EP: Evaluation of anxiolytic activity of spray dried powders of two South Brazilian Passiflora species. *Phys-
tother Res* 2006, 20(3):348-351.
15. Wheatley D: Medicinal plants for insomnia: a review of their pharmacology, efficacy and tolerability. *J Psychopharmacol* 2005, 19(4):414-421.
16. Akhondzadeh S, Mohammadi MR, Momeni F: Passionflora incarnata in treatment of attention-deficit hyperactivity disorder in chil-dren and adolescents. *Therapy* 2005, 2(4):609-614.
17. Ichimura T, Yamanaka A, Ichiba T, Toyokawa T, Kamada Y, Tama-
mura T, Maruyama S: Antihypertensive effect of an extract of *Passiflora edulis* rind in spontaneously hypertensive rats. *Bio-
sci Biotechnol Biochem* 2006, 70(3):718-721.
18. Rowe CA, Nantz MP, Deniera C, Green K, Talcott ST, Percival SS: Inhibition of neoplastic transformation of benzo[alpha]pyrene-treated BALB/c 3T3 murine cells by a phytochemical extract of passionfruit juice. *J Med Food* 2004, 7(4):402-407.
19. Dhawan K, Kumar S, Sharma A: Evaluation of central nervous system effects of *Passiflora incarnata* in experimental ani-mals. *Pharmaceutical Biology* 2003, 41(2):87-91.
20. Akhondzadeh S, Khashab I, Mohibseri M, Hosseini SH, Nikzad S, Khani M: Passionflower in the treatment of opiate withdrawal: a double-blind randomized controlled trial. *J Clin Pharm Ther* 2001, 26(5):369-373.
21. Fernandez SP, Wasowski C, Loscalzo LM, Granger RE, Johnson GA, Paladini AC, Marden M: Central nervous system depressant action of flavonoid glycosides. *Eur J Pharmacol* 2006, 539(3):168-176.
22. Soulimani R, Younus C, Jarmouni S, Bousta D, Misslin R, Mortier F: Behavioural effects of *Passiflora incarnata* L. and its indole alkaloid and flavonoid derivatives and maltol in the mouse. *J Ethnopharmacol* 1997, 57(1):11-20.
23. Vogel HG, Vogel WH: *Drug Discovery and Evaluation, Pharmacological Assay Berlin: Springer*; 1997.
24. Lee SE, Fellow S: Intrinsic actions of the benzodiazepine recep-
tor antagonist Ro 15-1788. *Psychopharmacology 1986, 88(1):1-11.*
25. Hosseinizadeh H, Parvareh S: Anticonvulsant effects of thymo-
quione, the major constituent of *Nigella sativa* seeds, in mice. *Phytotherapy* 2011, 1(1):56-64.
26. Hosseinizadeh H, Parvareh S, Nassiri-Ash M, Mansouri MT: Intracerebroventricular administration of thymoquinone, the major constituent of *Nigella sativa* seeds, suppress epileptic seizures in rats. *Med Sci Monit* 2005, 11(4):BR106-110.
27. Lauretti GR, Ahmad I, Pleuvry BJ: The activity of opioid analogues in seizure models utilizing N-methyl-D-aspartic acid, kainic
acid, bicuculline and pentylenetetrazole. Neuropharmacology 1994, 33(2):155-160.
28. Kaminski RM, Witsen JM, Shippenberg TS: Pharmacological and genetic manipulation of kappa opioid receptors: Effects on cocaine- and pentylenetetrazol-induced convulsions and seizure kindling. Neuropharmacology 2007, 52(3):895-903.
29. Shinomiya K, Inoue T, Utsu Y, Tokunaga S, Masuoka T, Ohmori A, Kamei C: Hypnotic activities of chamomile and Passiflora extracts in sleep-disturbed rats. Biol Pharm Bull 2005, 28(5):808-810.
30. Dhawan K, Kumar S, Sharma A: Anti-anxiety studies on extracts of Passiflora incarnata Linnaeus. J Ethnopharmacol 2001, 78(2-3):165-170.
31. Zanoli P, Avallone R, Baraldi M: Behavioral characterisation of the flavonoids apigenin and chrysin. Fitoterapia 2000, 71(Suppl 1):S117-S123.
32. Muller SD, Vasconcelos SB, Coelho M, Biavatti MW: LC and UV determination of flavonoids from Passiflora edulis medicinal extracts and leaves. J Pharm Biomed Anal 2005, 37(2):399-403.
33. Dhawan K, Kumar S, Sharma A: Comparative anxiolytic activity profile of various preparations of Passiflora incarnata linnaeus: a comment on medicinal plants standardization. J Altern Complement Med 2002, 8(3):283-291.
34. Riaz K, Honar H, Homayoun H, Rashidi N, Deghani M, Sadeghipour H, Gaskari SA, Dehpour AR: Sex and esterus cycle differences in the modulatory effects of morphine on seizure susceptibility in mice. Epilepsia 2004, 45(9):1035-1042.
35. Genoni E, Minghetti A: Neuropharmacological activity of extracts from Passiflora incarnata. Planta Med 1988, 54(6):488-491.
36. Yajima Y, Naito M, Takahashi-Nakano Y, Misawa M, Nagase H, Mizoguchi H, Tseng LF, Suzuki T: Effects of differential modulation of µ, δ- and κ-opioid systems on bicuculline-induced convulsions in the mouse. Brain Res 2000, 862(1-2):120-126.
37. Manocha A, Mediratta PK, Sharma KK: Studies on the anticonvulsant effect of US0488H on maximal electroshock seizure in mice. Pharmacol Biochem Behav 2003, 76(1):111-117.
38. Von Voigtlander PF, Hall ED, Ochoa MC, Lewis RA, Triezenberg HJ: U-54494A: a unique anticonvulsant related to kappa opioid agonists. J Pharmacol Exp Ther 1987, 243(2):542-547.
39. Becker A, Braun H, Schroder H, Grecksch G, Holtz V: Effects of enadoline on the development of pentylenetetrazol kindling, learning performance, and hippocampal morphology. Brain Res 1999, 823(1-2):191-197.
40. Rawls SM, McGinty JF: Kappa receptor activation attenuates L-trans- pyrroline-2, 4-dicarboxylic acid-evoked glutamate levels in the striatum. J Neurochem 1998, 70(2):626-634.
41. Wagner JJ, Caudle RM, Chavkin C: Kappa-opioids decrease excitatory transmission in the dentate gyrus of the guinea pig hippocampus. J Neurosci 1992, 12:132-141.

Pre-publication history
The pre-publication history for this paper can be accessed here:

http://www.biomedcentral.com/1472-6882/7/26/prepub