ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 8, Issue 1, 2015

ISSN - 0974-2441

Research Article

ACUTE TOXICITY STUDY OF (*Z*)-1-BENZHYDRYL-4- CINNAMYLPIPERAZINES IN SWISS ALBINO MICE

ANAXEE BARMAN, SHIVAPRAKASH S, LATHA DIWAKAR, REDDY GC*

Division of Biological & Chemical Sciences, Vittal Mallya Scientific Research Foundation, Bengaluru - 560 076, Karnataka, India.
Email: gcreddy@vmsrf.org

Received: 15 October 2014, Revised and Accepted: 12 November 2014

ABSTRACT

Objective: The objective was to study the acute toxicity of (Z)-1-benzhydryl-4-cinnamylpiperazine derivatives (1a-c) using Swiss Albino mice.

Methods: The acute oral and dermal toxicity studies were carried out based on OECD guidelines by adopting fixed dosage method (the limit dose is 2000 mg/kg body weight of test animal in case of dermal toxicity and 5000 mg/kg in oral toxicity). Toxicity and mortality rates were studied at intervals of 6 hrs and 14 hrs after administration of test compounds for 14 days.

Results: (Z)-1-benzhydryl-4-cinnamyl piperazine derivatives (1a-c) at oral treatment of 5000 mg/kg BW and dermal toxicity study of 2000 mg/kg BW did not show any pronounced toxicity indicating their potential use for therapeutic purposes.

Conclusion: All compounds 1a-c did not cause any mortality or changes in general behavior of the test animals at oral treatment of 5000 mg/kg BW indicating no conspicuous toxicity at the highest dose administered.

Keywords: (Z)-1-benzhydryl-4-cinnamyl piperazine derivatives, Acute toxicity, Albino mice, Cinnarizine, Flunarizine.

INTRODUCTION

In spite of tremendous advancement in the modern medicine, there are still a large number of ailments for which suitable drugs are yet to be found. In the present day, there is an urgent need to develop safer drugs for the treatment of various disorders. Toxicity study is one such test, which will examine toxic effects when a chemical is absorbed into the body, via mouth, skin, and lungs. Hence, toxicity test is essential parameter in identifying potential health hazards of any new compounds before being given to human beings with doses well above the expected therapeutic range.

(*E*)-1-benzhydryl-4-cinnamylpiperazines such as cinnarizine (stugeron), flunarizine and clocinizine are well-known histamine H1-receptor antagonists. They have selective calcium entry blocking activity and are widely used in cerebral and peripheral vascular disorders [1]. Cinnarizine and flunarizine are useful in the treatment of dizziness and are popular anti-vertiginous agents [2-4]. Flunarizine is reported as effective in the prevention of motion sickness [2] and also found to have dopamine antagonist characteristics [5,6].

Cinnarizine [7] and flunarizine were first synthesized by Janssen Pharmaceuticals in the year 1955 and 1968. The pharmacology and clinical studies of cinnarizine and flunarizine are well-established [8-12]. Though all pharmacological and toxicological studies were conducted on (*E*)-1-benzhydryl-4-cinnamylpiperazine, there is no report of any toxicity studies on (Z)-1-benzhydryl-4-cinnamylpiperazines (1a-c) (Fig. 1). However, (Z)-1-benzhydryl-4-cinnamyl piperazine derivatives have been found to exhibit anti-inflammatory and analgesic activities [13]. The present study is therefore, intended for evaluating the acute toxic effect of these compounds in laboratory animals.

METHODS

Chemicals

All the chemicals and solvents are of LR grade and obtained from Sigma-Aldrich, Merck, and Loba-chemie and used as such for the experiments.

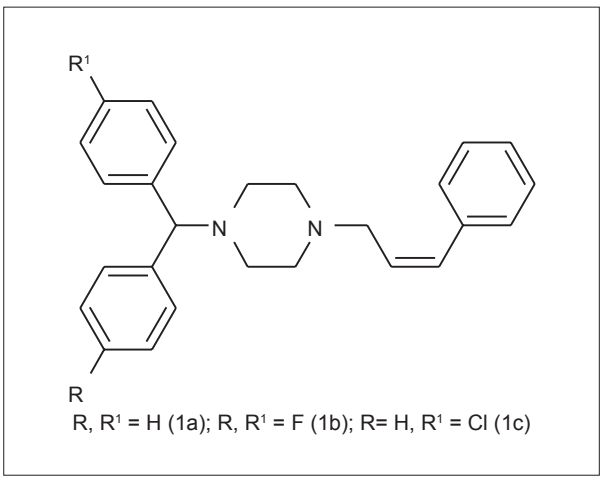

Fig. 1: The structure of (Z)-1-benzhydryl-4-cinnamylpiperazine derivatives

Test compounds

The (*Z*)-1-benzhydryl-4-cinnamylpiperazine derivatives (1a-c) were prepared according to our earlier procedure [14].

Animals

Experiments were performed using healthy young adult female Swiss albino mice, nulliparous, non-pregnant and weighing 25-30 g. Female mice were selected due to their greater sensitivity to treatment. The animals were housed in polypropylene cages (55 cm × 32.7 cm × 19 cm), in a temperature controlled environment (23±2°C). Lighting was controlled to supply 12 hrs of light and 12 hrs of dark for each 24 hrs period. Animal studies were approved by the Institute's Animal Ethical Committee (wide letter No VMSRF-14/IAEC/Oct 2013). The animals were taken care as per the CPCSEA guidelines, Ministry of Forests & Environment, Government of India.

Table 1: General appearance and behavioral observations for control and treated groups

Observation	Control group		Test group	
	6 hrs	14 hrs	6 hrs	14 hrs
Skin and fur	Normal	Normal	Normal	Normal
Eyes	Normal	Normal	Normal	Normal
Mucous membrane	Normal	Normal	Normal	Normal
Behavioural patterns	Normal	Normal	Rapid heart beat	Normal
Salivation	Normal	Normal	Normal	Normal
Lethargy	Normal	Normal	Normal	Normal
Sleep	Normal	Normal	Normal	Normal
Diarrhea	Normal	Normal	Normal	Normal
Coma	N.O	N.O	N.O	N.O
Tremors	N.O	N.O	N.O	N.O

N.O: Not observed

Acute oral toxicity study

The study was conducted in accordance with OECD guidelines (Testing of Chemical No. 423) [15]. 15 female Swiss albino mice weighing 25-30 g (three in each group) were randomly assigned into five groups and fasted for approximately 12 hrs prior to dosing. The test substance was formulated as a 200 mg/ml suspension in distilled water and administered at a dose of 5, 50, 300, 2000 and 5000 mg/kg body weight (BW) respectively to all the five groups by oral gavage. Each animal was observed for signs of toxicity at 6 hrs and 14 hrs after test substance administration, for 14 days. Mortality and morbidity checks were performed daily.

Acute dermal toxicity study

The study was conducted in accordance with the OECD guidelines (Testing of Chemical No. 402) [15]. 12 female mice (25-30 g) were randomly assigned to four groups (three in each group). The test substance was formulated as a 400 mg/ml suspension in distilled water then administered to each animal at the dose of 5, 50, 300, 2000 mg/kg BW. The fur on the back of each animal was closely clipped 24 hrs before treatment. The test substance was directly applied to a small area of skin. After application of the test substance, the test area was covered with a non-occlusive dressing (a gauze patch) and then a semi-occlusive bandage for 24 hrs. At the end of the exposure period, any residual test substance was removed by distilled water, and carefully dried. Each animal was observed for signs of toxicity once daily for 14 days following treatment. Mortality and morbidity checks were performed daily.

Statistical analysis

Data are presented as a mean±standard error of the mean. Comparisons were made between the treated groups by the use of single way Analysis of Variance. All data were analyzed using Sigmastat version 3.1 (Jandel Scientific Software, USA). $p<0.05$ was considered to be the level statistical significance.

RESULTS AND DISCUSSION

The toxic effect of (*Z*)-benzhydryl-4-cinnamylpiperazine derivatives (1a-c) on the appearance and the general behavioral pattern of mice are shown in Table 1. The result from the Table 1 indicated no toxic symptoms or mortality in any animals, which lived up to 14 days after the administration of the test compound at single dose level of 5000 mg/kg BW in acute oral toxicity study and also at dose level of 2000 mg/kg BW in acute dermal toxicity study. The behavioral patterns of animals were observed first 6 hrs and followed by 14 hrs after the administration and all animals were found normal and did not display any significant changes in behavior, skin effects, breathing, impairment in food intake and water consumption, postural abnormalities and hair loss. In the treated group in first 6 hrs rapid heartbeat was observed, but it then became normal and this may due to the stress of receiving the oral administration of the test substance.

The non-toxic nature of (Z)-1-benzhydryl-4-cinnamyl piperazine derivatives (1a-c) was evident by the absence of mortality of the test animals at oral treatment of 5000 mg/kg BW and dermal toxicity study of 2000 mg/kg BW.

CONCLUSION

In summary, the new compounds (Z)-1-benzhydryl-4-cinnamyl piperazine derivatives (1a-c) at oral treatment of 5000 mg/kg BW and dermal toxicity study of 2000 mg/kg BW did not show any conspicuous toxicity indicating their potential use as safe for therapeutic purposes. Hence, (Z)-1-benzhydryl-4-cinnamyl piperazine derivatives can be further exploited for anti-inflammatory and analgesic activities. A detailed experimental analysis of their chronic toxicities is essential for further support of these findings.

ACKNOWLEDGMENT

We thank Dr. Anil Kush, CEO, Vittal Mallya Scientific Research Foundation, for his encouragement and advice.

REFERENCES

1. Sweetman SC. Martindle, the Complete Drug Reference. 33rd ed. London: The Pharmaceutical Press; 2002. p. 413-8.
2. Lee JA, Watson LA, Boothby G. Calcium antagonists in the prevention of motion sickness. Aviat Space Environ Med 1986;57(1):45-8.
3. Verspeelt J, De Locht P, Amery WK. Postmarketing study of the use of flunarizine in vestibular vertigo and in migraine. Eur J Clin Pharmacol 1996;51(1):15-22.
4. Rascol O, Clanet M, Montastruc JL. Calcium antagonists and the vestibular system: A critical review of flunarizine as an antivertigo drug. Fundam Clin Pharmacol 1989;3 Suppl:79S-87.
5. Mena MA, Garcia de Yébenes MJ, Tabernero C, Casarejos MJ, Pardo B, Garcia de Yébenes J. Effects of calcium antagonists on the dopamine system. Clin Neuropharmacol 1995;18(5):410-26.
6. Micheli FE, Pardal MM, Giannaula R, Gatto M, Parera I, Paradiso G, *et al*. Movement disorders and depression due to flunarizine and cinnarizine. Mov Disord 1989;4(2):139-46.
7. Lucertini M, Mirante N, Casagrande M, Trivelloni P, Lugli V. The effect of cinnarizine and cocculus indicus on simulator sickness. Physiol Behav 2007;91(1):180-90.
8. Van Nueten JM, Janssen PA. Comparative study of the effects of flunarizine and cinnarizine on smooth muscles and cardiac tissues. Arch Int Pharmacodyn Ther 1973;204(1):37-55.
9. Barber JH, Reuter CA, Jageneau AH, Loots W. Intermittent claudication: A controlled study in parallel time of the short-term and long-term effects of cinnarizine. Pharmatherapeutica 1980;2(6):401-7.
10. Desmedt LK, Niemegeers CJ, Janssen PA. Anticonvulsive properties of cinnarizine and flunarizine in rats and mice. Arzneimittelforschung 1975;25(9):1408-13.
11. Schetz J, Bostoen H, Clement D, Fornhoff M, Haerens AR, Staessen AI. Flunarizine in chronic obstructive peripheral arterial disease. A placebo-controlled, double-blind, randomized multicentre trial. Curr Ther Res Clin Exp 1978;23:121-30.
12. Jan M, Paul MV. Flunarizine. In: Scriabine A, editor. New Drugs Annual: Cardiovascular Drugs. Vol. 2. New York: Raven Press; 1984.
13. Shivaprakash S, Burman A, Dewakar L, Reddy GC. *In vivo* studies of (*Z*)-1-benzhydryl-4- cinnamylpiperazines as anti-inflammatory and analgesics. Int J Pharm 2014;4(3):154-7.
14. Shivaprakash S, Reddy GC. Stereoselective synthesis of (*Z*)-1-benzhydryl-4- cinnamylpiperazines via the Wittig reaction. Synth Commun 2014;44:600-9.
15. OECD Guidelines for the Testing of Chemicals (No. 423) Acute Oral Toxicity-Acute Toxic Class Method [Adopted on 2011 Dec 17]. (b) OECD Guidelines for the Testing of Chemicals (No. 402) Acute Dermal Toxicity-Acute Toxic Class Method [Adopted on 2004 May 14]. (1st Version).