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In vivo studies of *(Z)*-1-benzhydryl-4- cinnamylpiperazines as anti-inflammatory and analgesics

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ABSTRACT

Benzhydryl cinnamyl piperazine drugs such as cinnarizine, flunarizine and clocinizine are being used as antihistamines and as calcium channel antagonists but possess *E*-geometry. The present study was designed to evaluate and compare anti-inflammatory and analgesic activities of *(Z)*-1-benzhydryl-4- cinnamylpiperazine derivatives (**2a-c**) with their *E* counterparts *in-vivo* using Swiss-albino mice. The results of the present study revealed that the Z-isomers have showed moderate to significant analgesic and anti-inflammatory action in a dose dependent manner. However, Z-counterpart of flunarizine exhibited remarkable analgesic property and also showed prolonged antiinflammatory activity compared to flunarizine.

Keywords: (*Z*)-1-benzhydryl-4- cinnamylpiperazine derivatives, Anti-inflammatory, Analgesic, albino mice

INTRODUCTION

Inflammation is caused due to response of living tissues for infection and injury. The main symptoms of inflammation include pain and swelling. [1] Most human diseases are associated with pain and inflammation component which lead to individuals seeking medical attention. [2] As such, analgesic and anti-inflammatory drugs are among the most prescribed drugs in clinical practice. [3] Despite the progress in the discovery of anti-inflammatory and analgesic drugs, the chronic use of these drugs is hampered by their adverse effects such as gastric lesions or tolerance as seen with NSAIDs and opiate analgesics respectively. Non steroidal anti-inflammatory agents [4-7] are known to reduce the production of prostaglandins that sensitize nerve endings at the site of injury. This effect occurs due to the inhibition of the cyclooxygenase (COX) enzyme that converts arachidonic acid liberated from the phospholipid membrane by phospholipases to prostaglandins. At least two forms of COX viz., COX1 and COX2 are thought to be important. COX1 is normally expressed in tissues such as stomach and kidneys and plays a physiologic role in maintaining tissue integrity. A second form, COX2, plays a role in pain and inflammation. [8] The analgesic effects of NSAIDs can be dissociated from anti-inflammatory effects, and this may reflect additional spinal and supraspinal actions of NSAIDs to inhibit various aspects of central pain processing. [9] Heterocyclic compounds containing piperazine moiety is considered as one of the main core nucleus which inhibit eicosanoid pathways [10] and possess different pharmacological properties [11] including anti-inflammatory, analgesic and antinociceptive activities.[12, 13] Currently there is a considerable therapeutic interest on novel drugs containing 1-arylpiperazinyl moieties. [14] The introduction of (4-arylpiperazin-1-yl) alkyl groups on various heterocyclic nuclei led to favourable antinociceptive compounds. [15-18]

Thus, piperazine derivatives are obviously attractive candidates for developing novel analgesic and anti inflammatory drugs. Hence, the present work was undertaken to evaluate the *(Z)*-1-benzhydryl-4-cinnamylpiperazines for their anti-inflammatory and analgesic activities in comparison to their *(E)* – geometrical isomers (Figure 1) by using experimental animal models.

MATERIALS AND METHODS

Animals: Male Swiss albino mice weighing about 22-25g were bred in our in-house animal facility. The protocol was approved by the institute's Animal Ethical Committee wide its letter No VMSRF-12/IAEC/Feb 2013. All the animals pertaining to the experiments were kept in disease free animal house at an ambient temperature at(delete) 25ºC and 45-55% relative humidity with 12h each of dark and light cycles. The animals had free access to food and drinking water as per CPCSEA dietary norms. The animals were acclimatized for at least 5 days to the laboratory conditions prior to experimentation. All the experiments were conducted between 09.00 to 18.00h.The animals were taken care as per the CPCSEA guidelines, Ministry of Forests & Environment, Government of India.

Test compounds: The compounds **1a-c** and **2a-c** were prepared according to our earlier procedure. [19] All the compounds were purified by passing through silica gel column before being taken for the experiment.

Experimental Design: Compounds **1a-c** and **2a-c** were assessed for anti-inflammatory and analgesic activities using carrageenan induced paw edema and acetic acid induced writhing in male mice respectively.

***Anti-inflammatory activity (Carrageenan induced paw edema in mice)*:** The anti-inflammatory activity was assessed by the method described by Wintar *et al.* [20] The mice were divided into 8 groups with 6 mice in each group. Paw swelling was elicited by sub-planter injection of 50µl of 1% sterile lambda carrageenan suspension in saline into the right hind paw. The contra lateral paw received an equal volume of saline. The paw volume was measured initially at 0, 2, and(delete) 4 and 24hours after carrageenan injection using a plethysmometer. The effect of cinnarizine, flunarizine and clocinizine at the dose rate of 10mg/kg, subcutaneous (s.c) was studied. The drug was administered 30min before the injection of carrageenan suspension. The difference between the initial and subsequent values gave the actual edema volume, which was compared with control. The percentage inhibition of inflammation was calculated using the formula

$$\frac{Vc - Vt}{Vc} \times 100$$

Where, Vc = edema volume in control; Vt = edema volume in group treated with test compounds

***Analgesic Activity (Acetic acid induced writhing reaction in mice)*:** The acetic acid induced writhing test in mice was employed as described by Koster et al. [21] Forty-eight Albino mice were divided into 8 groups of six mice each and were administered vehicle or drug @ dose rate of 10mg/kg s.c. After 30-min pretreatment interval, 0.6% acetic acid (0.2 ml/mice) was intraperitonially (i.p.) administered. The 0.6 % v/v solution of acetic acid was used as writhing inducing agents. Each mouse was then placed in an individual clear plastic chamber, and the total number of writhes made by each mouse was counted for 30 min after acetic acid administration. The numbers of abdominal contortions or writhing were counted for 30 min in control, standard and test compounds. The analgesic effect was assessed in each mouse and recorded. The degree of analgesia was calculated using the following formula (percentage inhibition of writhing)

$$\frac{\text{Mean of control group - Mean of treated group}}{\text{Mean of control group}} \times 100$$

Statistical analysis: Data analysis was carried out using one-way analysis expressed as mean ±SD. The data was analyzed using Bonferroni test. $p<0.0001$ was considered as statistical significance

RESULTS AND DISCUSSION

Anti-inflammatory Activity: The anti-inflammatory activity of *(Z)*-1-benzhydryl-4- cinnamylpiperazine compounds **(2a-c)** in comparison with their *E*-isomers **(1a-c)** on carrageenan induced paw edema have been shown in Table 1 as percentage inhibition at different time intervals. As could be seen that most of the compounds exhibited certain inhibitory effect with respect to standard diclofenac sodium in dose dependent manner. Cinnarizine **(1a)** was found have a significant ($p<0.0001$) anti-inflammatory effect when compared to its *Z*-isomer **(2a).** However, the inhibition effect of **2a** was better than **1a** at 24hr. Similarly, compound **2c** showed better inhibition at 24hr as against to its *E*-isomer **(1c).** In the case of compound **2b,** the Z-geometry 'was' may be replaced with 'showed' comparatively significant inhibitory effect than to(delete) its *E*-isomer **(1b)** at different time intervals.

Analgesic activity: The analgesic activity of (*Z)*-1-benzhydryl-4- cinnamylpiperazine compounds **(2a-c)** in comparison with their *E*-isomers **(1a-c)** on acetic acid induced writhing in mice have been shown in table 2 .All the compounds exhibited moderate to significant inhibitory effects against standard

diclofenac sodium. While(delete) Antiinflammatory activities of *E*-isomers in general are lower than their *Z*- counterparts. As seen in table 2, *Z*-isomers **2a**, and **2c** were found to have lower analgesic activity when compared with their *E*-isomers **1a** and **1c**, but in the case of compound **2b,** the analgesic effect was better than its *E*- derivative **(1b).**

CONCLUSION

From our overall experimental results, it is concluded that most of the tested compounds exhibited moderate to good anti-inflammatory and analgesic activities against standard drug diclofenac sodium. In general, *E*-isomers exerted better activity when compared to their *Z*-compounds. However, Z- isomer of compound 2b showed better anti-inflammatory effect than of its *E*-compound 1b. The analgesic effect of compound 2b was comparatively better than the standard drug diclofenac sodium and almost equipotent to cinnarizine (1a). Hence compound 2b can be considered as a useful compound for the treatment of pain and inflammatory diseases.

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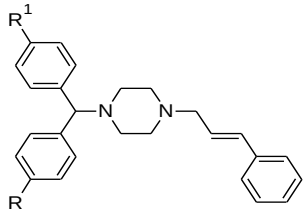
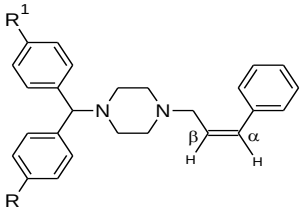


R, R^1 = H (Cinnarizine) **(1a)**; R, R^1 = F (Flunarizine) (**1b**); R = H, R^1 = Cl (Clocinizine) (**1c) (***E*-isomers)



R, R^1 = H (*cis*-Cinnarizine) **(2a)**; R, R^1 = F (*cis*-Flunarizine) (**2b**); R=H, R^1 = Cl (*cis*-Clocinizine) (**2c)** (*Z*-isomers)

Figure 1. Chemical structure of 1-benzhydryl-4-cinnamylpiperazine derivatives

Table 1: Anti-inflammatory activity of compounds 1a-c and 2a-c on carrageenan induced paw edema in mice

Treatment (10mg/kg)	Paw edema in mL				% Inhibition of paw edema			
	0hr	2hr	4hr	24hr	0hr	2hr	4hr	24hr
Cinnarizine **(1a)**	0.37± 0.029	0.32± 0.025	0.24± 0.016*	0.29± 0.023*	6.96	27.16	27.61	1.92
Flunarizine **(1b)**	0.36± 0.023	0.31± 0.032*	0.25± 0.031*	0.30± 0.034*	0.52	13.46	7.69	13.91
Clocinizine **(1c)**	0.35± 0.025	0.24± 0.033*	0.25± 0.024*	0.35± 0.021	10.3 1	42.79	27.88	9.13
cis-Cinnarizine **(2a)**	0.36± 0.011	0.34± 0.021	0.31± 0.019*	0.33± 0.022	5.16	6.01	11.73	4.83
cis-Flunarizine **(2b)**	0.37± 0.034	0.34± 0.047	0.33± 0.049	0.36± 0.031	3.35	21.88	30.77	0.48
cis-Clocinizine **(2c)**	0.38± 0.015	0.36± 0.025	0.33± 0.026	0.31± 0.028	5.67	18.27	4.81	16.31
Diclofenac sodium (standard)	0.23± 0.021	0.33± 0.022	0.20± 0.011	0.19± 0.013	7.0	42.16	66.21	69.94
Positive control / Carrageenan	0.38± 0.038	0.42± 0.042	0.43± 0.031	0.35± 0.032	-	-	-	-

Values are expressed as Mean ± S.D $^{*}p<0.000$ (this line should go up)

Table 2: Analgesic activity of compounds 1a-c and 2a-c on acetic acid induced writhing in mice

Treatment(10mg/kg)	No of writhing	Percentage inhibition
Cinnarizine **(1a)**	25.16 ± 3.430	78.14
Flunarizine **(1b)**	11.33 ± 1.861	65.27
Clocinizine **(1c)**	18.0 ± 2.366	76.85
cis-Cinnarizine **(2a)**	51.83 ± 8.084	59.17
cis-Flunarizine **(2b)**	21.16 ± 2.136	78.78
cis-Clocinizine **(2c)**	11.0 ± 1.414	51.45
Diclofenac sodium (standard)	18.14 ± 1.18	75.78

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