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Analgesic and anti-inflammatory activities of *Annona squamosa* Linn bark

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Abstract

Caryophyllene oxide was isolated from a methanolic extract of the bark of *Annona squamosa* and studied for its analgesic and anti-inflammatory activity. Caryophyllene oxide at the doses of 12.5 and 25 mg/kg body wt. and methanol extract at a dose of 50 mg/kg body wt. showed significant central as we as peripheral analgesic along with anti-inflammatory activity. These activities of caryophyllene oxide were comparable with the standard drug Pentazocin (50 mg/kg body wt.) for Anti-inflammatory activities and Aspirin (100 mg/kg body wt.) for Analgesic activity used in the respective experiments.

Keywords: *Annona squamosa*, Methanolic extract, Caryophyllene oxide, Analgesic, Anti-inflammatory.

Introduction

Annona squamosa L. (Annonaceae) is a tree occurs wild and is also cultivated throughout India. Its leaves are used as insecticidal and antispasmodic agents and are used in the treatment of rheumatism and painful spleen. The plant is reported to possess analgesic, anti-inflammatory[1], antipyretic, antiulcer, and antiseptic and abortifacient activities[2]. Its use as an insecticidal agent has been investigated by several workers[3] and various phytochemical, pharmacological, antibacterial and antiovulatory studies have already been carried out with the seed extracts[4]. Post-cortical antifertility activity of *A. squamosa* has also been reported from studies with the seed extract.[5]

A tetrahydroisoquinoline alkaloid with cardiotonic activity has been isolated from the leaves of *Annona squamosa1*[1] along with a bioactive acetogenin from its bark[6]. A novel diazepine, squamolone was also isolated from this plant.[7] The purpose of the study reported here was to isolate the pure constituent responsible for analgesic and anti-inflammatory activity from the bark of the plant.

Materials and Methods

Plant material

Bark of *Annona squamosa* L. was collected from Banglore district, Karnatka, in August 2008.

Extraction and isolation

Shade dried powdered material weighing 1kg was extracted by soxhlet using methanol.

The solvent was evaporated in vacuum to obtain the crude methanol extract. The methanolic extract (ME) (10 g) obtained was applied to a column of Silica gel 60 (60-120 mesh) packed in benzene slurry and the column was developed with benzene, from which were collected 14 fractions of 300-400 ml each. Fractions 6-10 were combined on the basis of similar TLC pattern (Si gel plates, benzene, vanilline-sulphuric acid spray). These fractions were further resolved by preparative TLC on silica gel using benzene as a mobile phase, resulting in isolation of two minor compounds along with caryophyllene oxide (32 mg). Caryophyllene oxide: white amorphous powder, m.p. 60-61 ºC. NMR and mass spectra of the compound were matched with those reported in the literature.[2]

Animals

All the experiments were carried out using male Swiss albino mice (20-25 g each) and Wistar rats (150-200 g each). The animals had free access to food and water and they were housed under a natural (12 h each) light-dark cycle with access to standard pellet chow and water ad libitum. The animals were acclimatized for at least 5 days to the laboratory conditions before performing the experiments. The experimental protocol was approved by the Institutional Animal Ethics Committee (Registration No. 372/01/a/ CPCSEA). In all experimental models, six animals were used in each group.

Analgesic activity

The central analgesic activity of the test drug was studied against thermal stimuli using the hot plate test[8], while peripheral analgesic activity of the test drug was evaluated using the acetic acid-induced writhing test[9].

Eddy's hot plate test

The initial reaction times of all the animals of control and test groups were recorded by putting them on the hot plate maintained at 55 ± 0.5ºC. Licking of paw or jumping was taken as the index of reaction to heat. The albino mice were divided into five groups. ME (50 mg/kg body wt.), Caryophyllene oxide (12.5 and 25 mg/kg body wt.), or pentazocin lactate injection (50 mg/kg body wt.) were administered by intra peritoneal route. The first group served as control and received vehicle only (1% dimethyl formamide in water for injection). The post-treatment reaction time of each animal was recorded at 30, 60, 90, 120 and 180 min.

Acetic acid induced writhing

The albino mice were divided into five groups. ME (50 mg/kg body wt.), Caryophyllene oxide (12.5 and 25 mg/kg body wt.) or aspirin (100 mg/kg body wt.) were administered one hour prior to intra peritoneal injection of 0.6% v/v acetic acid. Five minutes after the intra peritoneal injection of acetic acid, the number of writhings during the following 20 min was counted. Control mice received vehicle only (1% dimethyl formamide in water for injection).

Anti-inflammatory activity

Albino rats of Wistar strain of either sex were divided into five groups. Acute inflammation was produced by sub plantar injection of 0.1 ml of 1% suspension of carrageenan with 2% gum acacia in normal saline, in the right hind paw of rats, one hour after oral administration of ME (50 mg/kg body wt.), Caryophyllene oxide (12.5 and 25 mg/kg body wt.) or aspirin (100 mg/kg body wt.). Control rats were received vehicle only (1% dimethyl formamide in water for injection). The paw volume was measured plethysmometrically (Ego Basil, Italy) at 0, 1, 2, and 3 h after the carrageenan injection. The difference between '0' readings and readings after 1, 2, and 3 h, respectively, was taken as the volume of edema.[10]

Statistical Analysis

All the results were statistically analyzed by student's t-test and expressed as mean ± S.E.M. Results were regarded as significant when $p<0.05$.

Results

The effect of Methanolic extract and pure caryophyllene oxide were evaluated for central as well as peripheral analgesic, along with anti-inflammatory, activity. Table 1 shows the results on thermic stimulus induced pain (Eddy's hot plate test) in mice. Pretreatment with pentazocin or ME or caryophyllene oxide did not produce any significant changes of paw licking time in the early phase of pain. However, in the late phase, a dose-dependent and significant ($p<0.05$) increase in licking time was observed in mice treated with caryophyllene oxide as well as with ME and pentazocin. The maximum activity was observed with caryophyllene oxide (25 mg/kg body wt., i.p.) at the 120 min time interval, which is comparable to the standard pentazocin. The maximum analgesia induced by ME (50 mg/kg body wt., i.p.) was at the 60 min time interval and persisted up to 120 min.

Table 2 shows the response of mice to acetic acid-induced writhing. Treatment of M E, caryophyllene oxide and standard aspirin significantly ($p<0.05$) reduce the number of writhes. The inhibitions were 64.89%, 57.87% and 75.19% for ME and two doses of pure caryophyllene oxide, respectively. At the dose of 25 mg/kg body wt., caryophyllene oxide inhibited the writhing response almost to the same degree as aspirin (74.41%). Table 3 shows the response of rats to the carrageenan induced paw edema. The results obtained with ME, caryophyllene oxide and standard aspirin show significant ($p<0.05$) inhibition of inflammatory edema at the first and second hours after the carrageenan treatment. The effect of caryophyllene oxide at a dose of 25 mg/kg body wt. p.o. was comparable to standard aspirin. The dose-dependent inhibition of edema was observed with caryophyllene oxide treatment.

Table 1: Effect of Caryophyllene oxide on thermic stimulus-induced pain in mice (hot plate test)

Treatment	Pre drug reaction time	30 min	60 min	90 min	120 min	180 min
Control	12.45 ± 0.50	8.50 ± 0.92	9.00 ± 1.15	8.83 ± 0.54	8.16 ± 1.10	8.00 ± 0.89
Pentazocin (50 mg/kg body wt.)	8.83 ± 0.87	16.16 ± 1.24 *	8.83 ± 0.87	16.16 ± 1.24 *	17.50 ± 0.95 *	7.08 ± 0.58
ME (50 mg/kg body wt.)	20 ± 0.18 *	20 ± 0.94 *	20 ± 0.18 *	20 ± 0.94 *	20 ± 0.26 *	10.74 ± 0.20
Caryophyllene oxide (12.5 mg/kg body wt.)	8.92 ± 1.2	6.66 ± 0.88	10.33 ± 0.95*	12.33 ± 1.20 *	14.5 ± 1.02 *	8.00 ± 0.68
Caryophyllene oxide (25 mg/kg body wt.)	9.45 ± 0.94	5.50 ± 0.76	8.33 ± 0.91	12.66 ± 0.98 *	17.0 ± 0.96 *	9.33 ± 0.80

Values are mean ± S.E.M., n= 6, * $p<0.05$, significant* compared to control. ME- Methanolic Extract

Table 2: Effect of Caryolphyllene oxide on acetic acid-induced writhing test in mice

Treatment	No. of writhing	Inhibition (%)
Control	42.33 ± 1.62	-
Aspirin (100 mg/kg body wt.)	10.83 ± 0.65 *	74.41
ME (50 mg/kg body wt.)	14.86 ± 1.21 *	64.89
Caryophyllene oxide (12.5 mg/kg body wt.)	17.83 ± 1.10 *	57.87
Caryophyllene oxide (25 mg/kg body wt.)	10.50 ± 1.05 *	75.19

Values are mean ± S.E.M., n = 6. * $p<0.05$, significant * compared to control. ME- Methanolic extract

Table 3: Effect of Caryophyllene oxide on carrageenan-induced paw edema in rats

Treatment	Increase in paw volume in ml for		
	1 Hour	2 Hours	3 Hours
Control	0.56 ± 0.036	0.55 ± 0.029	0.65 ± 0.064
Aspirin (100 mg/kg body wt.)	0.23 ± 0.017 *	0.20 ± 0.011 *	0.66 ± 0.036
ME (50 mg/kg body wt.)	0.26 ± 0.016 *	0.29 ± 0.024 *	0.61 ± 0.028
Caryophyllene oxide (12.5 mg/kg body wt.)	0.25 ± 0.016 *	0.30 ± 0.021 *	0,45 ± 0.028 *
Caryophyllene oxide (25 mg/kg body wt.)	0.23 ± 0.015 *	0.27 ± 0.013 *	0.66 ± 0.036

Values are mean ± S.E.M., n=6, * <0.05, significant * compared to control. ME-Metrhanolic Extract

Discussion

The results of the present study show that the methanolic extract and caryophyllene oxide isolated from this extract of Annona squamosa bark exhibit significant analgesic and anti-inflammatory activities. The results obtained in the analgesic test experiments appear to suggest that ME and pure caryophyllene oxide isolated from this extract possess centrally and peripherally mediated analgesic properties. The central analgesic action may be mediated via inhibition of central pain receptors, while the peripheral analgesic effect may be mediated through inhibition of cyclooxygenase and/or lipoxygenase (and other inflammatory mediators). This hypothesis is in consonance with those of Eddy *et al*[8], Koster *et al*[9] and Wagner *et* al[11] who postulated that acetic acid writhing and hot plate test methods are useful techniques for the evaluation of centrally and peripherally acting analgesic drugs, respectively. Caryophyllene oxide produced an analgesic effect against thermal induced pain stimuli in mice at various time points post-treatment. The effect observed was dose-dependent and statistically significant. The hot plate test is considered to be selective for opioid-like compounds, which are centrally acting analgesics in several animal species.[12] Furthermore, the effect of ME was initiated first and appears for long duration when compared with different doses of caryophyllene oxide in the hot plate test. This might be due to the other constituents present in the ME, such as the kaurane-type diterpenes, which might contribute to the central analgesic activity.[6]

The carrageenan-induced rat paw edema is a suitable test for evaluating anti-inflammatory drugs that has frequently been used to assess the anti-edematous effect of natural products.[5] Development of the edema in the paw of the rat after injection of carrageenan is a biphasic event. The initial phase observed during the first hour is attributed to the release of histamine and serotonin. The second phase of edema is due to the release of prostaglandins, protease and lysosomes.[13, 14] The ME at 50 mg/kg body wt. and caryophyllene oxide at doses of 12.5 and 25 mg/kg body wt. shows significant activity in the first and second hours. This might be due to the inhibition of the biphasic response induced by the carrageenan.

Conclusion

Caryophyllene oxide administered intra peritoneally exhibits antinociceptive activity and might exert its effect through diverse mechanisms that may involve both central and peripheral pathways. Further pharmacodyanamic investigations are required to understand the analgesic and anti-inflammatory activity exhibited by caryophyllene oxide.

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