Studies on the Antiinflammatory, Antipyretic and Analgesic Activities of Santonin

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ABSTRACT—Santonin, a sesquiterpene lactone, commonly found in the plants of the family *Compositae* was found to show significant antiinflammatory activity on acute inflammatory processes. The activity profile of santonin closely resembled that of a standard non-steroidal antiinflammatory drug, diclofenac sodium. It also showed a significant inhibitory effect on granuloma formation; however, this effect of santonin was less pronounced as compared to diclofenac sodium. Santonin caused a significant antipyretic effect in mice, which was found to be independent of the route of administration of the drug. It also increased the hot plate reaction time of treated mice, similar to morphine.

Keywords: Santonin, Antiinflammatory, Antipyretic, Analgesic

Artemisia species (*Compositae*) are known for their antibacterial, anthelmintic, antiinflammatory, stimulatory and antispasmodic activities (1-4). The interperitoneal administration of santonin, one of the major compounds isolated from these plants (3) was found to cause a significant antipyretic effect in rats (5). So far, no report is available on the antiinflammatory activity of santonin. However, it induced a mild inhibitory effect on the cytotoxicity of MM2 tumor cells of polymorphonuclear leucocytes (PMN) induced by TAK (a polysaccharide immunomodulator). Several natural antiinflammatory compounds were found to show such inhibition (6). In a recent report, the antiinflammatory activity of *A. inculta* extract, without testing any pure compound, was associated with the presence of flavonoids and sesquiterpene lactones in the extract (4). Several flavonoids (7-10) and some oxygenated sesquiterpenes and sesquiterpene hydrocarbons (11-13) are known for their antiinflammatory potential. In the light of the available literature, there is a need to explore different properties of santonin and related terpenoids that may help in understanding the mechanism of the antiinflammatory activity common to several *Compositae* plants. The present study was designed to determine the antiinflammatory potential of santonin. Furthermore, the antipyretic and analgesic activities of santonin were also explored by changing the route of administration and animal models. The results are presented in this communication.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing 180-200 g and Swiss albino mice (SWR, home bred) weighing 25-30 g were used in the present study.

Drugs

Santonin (Winlab Ltd., Maidenhead, UK); diclofenac sodium (Ciba Geigy Ltd., Basle, Switzerland); carrageenan sodium salt, yeast, morphine (BDH Chemicals Ltd., Poole, UK) and sodium salicylate (E. Merck AG, Darmstadt, Germany) were used.

Carrageenan-induced paw edema in rats

Inflammation in rat paw was produced by the method described by Winter et al. (14). The test group of rats was treated with santonin (15, 30, 60 and 120 mg/kg body weight). The animals in the reference control group were administered diclofenac sodium (a standard non-steroidal antiinflammatory drug) at a dose of 75 mg/kg. The control group of rats was given distilled water. The drugs in each case were dissolved in water and administered orally

1 hr before carrageenan injection, and the paw volume was measured at 1, 2, 3 and 4 hr after the carrageenan injection. Inhibition of inflammation was calculated according to the following formula:

$$\text{Percentage inhibition} = \left(1 - \frac{a-x}{b-y}\right) \times 100$$

where 'x' and 'a' are the mean paw volume of the rats before and after the administration of carrageenan, respectively, in the test or reference control group, whereas 'y' and 'b' are the mean paw volume of rats before and after the administration of carrageenan in the control group.

Cotton pellet granuloma in rats

The method of Goldstein et al. (15) was used with slight modification. A sterilized cotton pellet weighing 30 mg was introduced in the groin region of rats in different groups. They were treated with santonin (15, 30, 60 and 120 mg/kg) daily for five consecutive days, whereas distilled water was given to the control animals. Diclofenac sodium (30 mg/kg) was used as a standard antiinflammatory drug. On the sixth day, the animals were killed under ether anesthesia. Then the pellets were removed; and after removing extraneous tissues, they were dried overnight at 60℃ and weighed.

Antipyretic activity in mice

Hyperpyrexia was produced in mice by s.c. administration of 2 ml/100 g of 20% aqueous suspension of brewer's yeast (16). The animals were then fasted for the entire duration of the experiment. The pyretic response to yeast was measured approximately within 24 hr of the induction of hyperpyrexia. The aqueous solution of santonin was administered at a dose of 15, 30, 60 and 120 mg/kg, and rectal temperatures were recorded at 30, 60 and 90 min post treatment of santonin. Sodium salicylate (300 mg/kg) was used as a standard antipyretic drug.

Analgesic activity in mice

The hot plate method described by Turner was used (17). The mice were dropped on a hot plate maintained at 55±0.5℃. The reaction time was taken as the interval from the instant the animal reached the hot plate until the moment the animal licked its feet or jumped out. The reaction time was measured at 30, 60, 90 and 120 min after the administration of the drug. The animals in the test groups were given an aqueous solution of santonin at a dose of 15, 30, 60 and 120 mg/kg, and morphine (10 mg/kg) was used as a standard analgesic drug.

RESULTS

Effect on carrageenan-induced paw edema in rats

The pretreatment with santonin resulted in a significant and dose-dependent reduction in carrageenan induced paw edema in rats except at 15 mg/kg. The percent inhibition was comparatively less at 1 hr after treatment with lower doses of santonin (30 and 60 mg/kg) when compared to the effect of santonin at 120 mg/kg and diclofenac sodium at 75 mg/kg. The maximum inhibition of paw volume was observed at 2 hr after treatment with santonin (30–120 mg/kg). However, the reduction at 3 and 4 hr after santonin treatment (120 mg/kg) was also close to the effect of diclofenac sodium (Table 1).

Antigranulation effect in rats

The results of the effect of santonin on granuloma formation in the cotton pellet method are presented in Table 2. Santonin treatment caused 42% inhibition at 60 mg/kg and 55% inhibition at 120 mg/kg. There was no significant effect by santonin at 15 and 30 mg/kg treatment. The standard drug diclofenac sodium caused 59% inhibition at a relatively lower dose level (30 mg/kg) as compared to the control.

Table 1. Effect of santonin on carrageenan-induced rat paw edema

Treatment and dose (mg/kg, p.o.)	Total increase in paw volume (mean±S.E.) (percent inhibition)			
	1 hr	2 hr	3 hr	4 hr
1. Control (D.W.)	0.33±0.03	0.70±0.04	0.99±0.05	1.07±0.04
2. Santonin (15)	0.28±0.04 (15)	0.41±0.02 (13)	0.86±0.06 (13)	0.96±0.04 (10)
3. Santonin (30)	0.23±0.02* (30)	0.31±0.04*** (55)	0.63±0.05*** (36)	0.77±0.02*** (29)
4. Santonin (60)	0.19±0.03* (42)	0.24±0.02*** (65)	0.48±0.04*** (51)	0.55±0.04*** (29)
5. Santonin (120)	0.14±0.02*** (57)	0.19±0.02*** (73)	0.38±0.03*** (61)	0.46±0.03*** (57)
6. Diclofenac sodium (75)	0.09±0.02*** (72)	0.10±0.02*** (85)	0.29±0.03*** (70)	0.38±0.01*** (64)

D.W.=Distilled water. Five animals were used in each group. Groups 2, 3, 4, 5 and 6 were statistically compared with group 1; *$P<0.05$ and ***$P<0.001$ (Student's *t*-test). The effect of santonin alone (15–120 mg/kg) on the paw volume of untreated rats was negligible.

Table 2. Effect of santonin on cotton pellet granuloma formation in rats

Treatment and dose (mg/kg/day, p.o.)	Original weight of pellet in mg (mean±S.E.)	Weight increased in mg (mean±S.E.)	Percent inhibition
1. Control (D.W.)	30.01±0.02	49.72±4.75	—
2. Santonin (15)	30.04±0.03	45.36±3.45	9
3. Santonin (30)	30.06±0.04	39.23±4.00	21
4. Santonin (60)	30.08±0.02	28.46±3.27	42*
5. Santonin (120)	30.10±0.08	22.37±4.45	55*
6. Diclofenac sodium (30)	30.04±0.02	20.35±1.83	59***

D.W.=Distilled water. Five animals were used in each group. Groups 2, 3, 4, 5 and 6 were statistically compared with group 1; *P<0.05 and ***P<0.001 (Student's *t*-test).

Antipyretic activity in mice

The results presented in Tables 3 and 4 show the antipyretic activity of santonin. The oral administration of santonin (15–120 mg/kg) caused a dose-dependent reduction in the body temperature of normal mice. The subcutaneous injection of brewer's yeast considerably elevated the rectal temperature of mice. This rise in temperature was decreased significantly in a dose-dependent manner after oral administration of santonin (15–120 mg/kg). The effect was clear by 30 min after administration and remained stable up to 90 min. A similar antipyretic effect was shown by the reference drug sodium salicylate at a relatively higher dose (300 mg/kg).

Analgesic activity in mice

The results presented in Table 5 show that santonin significantly increased the hot plate reaction time of mice up to 60 min at a dose of 60 and 120 mg/kg. However, there was a decline in the reaction time beyond 90 min. A similar effect was observed by morphine (10 mg/kg, i.p.) injection.

DISCUSSION

The present study shows that santonin possesses antiinflammatory activity on carrageenan-induced edema in rat paw. The activity profile of santonin closely resembled that of diclofenac sodium. Both the drugs induced a maximal inhibitory effect at 2 hr following the injection of carrageenan and remained steady during the following hours. As reported earlier (18, 19), the process of carrageenan-induced paw edema in rats involves three

Table 3. Effect of santonin on the body temperature of normal mice

Treatment and dose (mg/kg, p.o.)	Pre-drug rectal temp. ℃ (mean±S.E.)	Post-drug rectal temperature ℃ (mean±S.E.)		
		30 min	60 min	90 min
1. Control (D.W.)	36.61±0.31	36.48±0.32	36.42±0.42	36.40±0.36
2. Santonin (15)	36.49±0.32	35.96±0.39	35.26±0.28*	36.30±0.52
3. Santonin (30)	36.51±0.38	34.08±0.48**	33.21±0.20***	32.72±0.31***
4. Santonin (60)	36.58±0.29	34.01±0.31***	33.18±0.17***	33.15±0.19***
5. Santonin (120)	36.45±0.34	33.82±0.29***	32.45±0.21***	32.32±0.34***

D.W.=Distilled water. Five animals were used in each group. In each group, the post-drug rectal temperatures at 30, 60 and 90 min were statistically compared with the pre-drug rectal temperature; *P<0.05, **P<0.01 and ***P<0.001 (Student's *t*-test).

Table 4. Effect of santonin on yeast induced hyperthermea in mice

Treatment and dose (mg/kg, p.o.)	Pre-drug rectal temp. ℃ (mean±S.E.)	Post-drug rectal temperature ℃ (mean±S.E.)		
		30 min	60 min	90 min
1. Control (D.W.)	38.10±0.19	38.18±0.27	38.16±0.26	38.34±0.28
2. Santonin (15)	38.64±0.27	34.56±0.50***	34.30±0.38***	35.24±0.87**
3. Santonin (30)	38.46±0.12	34.36±0.16***	34.20±0.73***	34.36±0.42***
4. Santonin (60)	37.72±0.22	33.90±0.27***	33.90±0.20***	33.52±0.24***
5. Santonin (120)	38.04±0.09	33.70±0.28***	32.88±0.40***	33.00±0.43***
6. Sodium salicylate (300)	37.80±0.16	36.20±0.14***	36.30±0.17***	36.38±0.16***

D.W.=Distilled water. Five animals were used in each group. In each group, the post-drug rectal temperatures at 30, 60 and 90 min were statistically compared with the pre-drug rectal temperature; **P<0.01 and ***P<0.001 (Student's *t*-test).

Table 5. Effect of santonin on hot plate reaction time in mice

Treatment and dose (mg/kg, p.o.)	Pre-drug reaction time in seconds (mean ± S.E.)	Post-drug reaction time in seconds (mean ± S.E.)			
		30 min	60 min	90 min	120 min
1. Control (D.W.)	11.44 ± 1.52	12.57 ± 1.92	12.64 ± 2.09	11.58 ± 2.00	11.25 ± 1.60
2. Santonin (15)	13.19 ± 2.02	8.82 ± 0.80	15.98 ± 2.46	11.78 ± 1.29	10.31 ± 0.52
3. Santonin (30)	12.11 ± 2.32	12.36 ± 2.34	15.29 ± 2.06	13.13 ± 1.88	11.96 ± 1.75
4. Santonin (60)	11.69 ± 0.64	12.99 ± 0.46	15.32 ± 1.34*	11.62 ± 0.88	11.15 ± 1.08
5. Santonin (120)	12.06 ± 0.95	23.92 ± 2.83**	16.65 ± 1.13*	11.70 ± 0.94	9.68 ± 1.09
6. Morphine (10, i.p.)	12.09 ± 1.28	16.35 ± 2.40	18.81 ± 1.20**	13.24 ± 1.23	8.82 ± 0.34*

D.W. = Distilled water. Five animals were used in each group. In each group, the post-drug reaction times at 30, 60, 90 and 120 min were statistically compared with the pre-drug reaction time; *$P < 0.05$ and **$P < 0.01$ (Student's *t*-test).

phases. The higher activity of santonin observed during the second and third phases of inflammation suggests that the activity may be due to the suppression of kinins and prostaglandins formation induced by carrageenan within these periods (19). The antiinflammatory effect of santonin is similar to that of diclofenac sodium, an inhibitor of cyclooxygenase (20).

To further verify the antiinflammatory activity of santonin and its effects on the proliferative phase of inflammation, the cotton pellet granuloma bioassay model was used. At the doses used in this model, santonin showed a significant inhibitory effect on granuloma formation. This study revealed that santonin was active against the inflammation induced by a foreign body as well, although to a much lesser degree than plantar edema. This effect of santonin was, however, less pronounced than that of diclofenac sodium. Kinoshita et al. (6) showed that santonin may cause inhibition of the TAK (a linear B-1, 3-D-glucan)-induced PMN activation like some other natural antiinflammatory drugs. Several reports suggested that plant extracts rich in sesquiterpenoids, viz. *Siegesbeckia pubescens* (7), *Conzya canadensis* (7), *Commiphora molmol* (21), *Artemisia inculta* (4), *Baldwina augustifolia* (7), *Tanacetum corymbosum* (22) and *Vanillosmopsis araborea* (23), possess a varied range of antiinflammatory activity. Sesquiterpenoids also possess the ability to stabilize lysosomal membrane and cause significant anticoagulant and antifibrinolytic effects (22). Based on the results of our present study, it appears that santonin achieves its antiinflammatory effect by inhibiting kinins and prostaglandins biosynthesis and due to its possible anticoagulant and antifibrinolytic properties.

According to the findings of the present experiment, santonin oral administration was found to decrease the body temperature of normal mice and was confirmed to show a significant antipyretic effect in yeast-fevered mice. It has been postulated that the hypothalamic regulation of body temperature takes place through chemical neuromediators such as dopamine, acetylcholine and serotonin. Previously, santonin i.p. administration was found to cause an antipyretic effect that was inhibited by haloperidol (5), confirming the participation of a hypothalamic neuromediator of the dopamine type. Our findings are in agreement with the earlier reports (5) and further add that santonin achieves its antipyretic effect independent of the route of administration.

The present study also revealed that santonin considerably increased the hot plate reaction time of the treated animals. Santonin is known to produce a central depressive action (5). The effect observed during the present study was found to be similar to that of morphine, which further indicated the possibility that santonin may have central analgesic properties.

The results of our present study clearly demonstrated the strong antiinflammatory, antipyretic and analgesic properties of santonin. However, further studies are warranted to ellucidate the exact mode of action and safety of this potent drug.

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