STUDIES ON THE ANTI-INFLAMMATORY ACTIVITY OF MOSCHUS MOSCHIFERUS (MUSK) AND ITS POSSIBLE MODE OF ACTION

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Summary: *Moschus moschiferus* (musk) had significant anti-inflammatory activity in carrageenin-induced oedema, formaline arthritis and adjuvant arthritis. It was more potent than hydrocortisone and phenyl-butazone in adjuvant arthritis. The secondary lesions produced in adjuvant arthritis were mild in musk and phenylbutazone-treated groups in comparison to the moderate lesions in hydrocortisone-treated group. Also, the weight loss was less in musk and phenylbutazone-treated groups. Musk failed to prolong the survival time of the adrenalectomized rats. It significantly decreased the histamine and the 5-HT content of the inflammed paw of the rats. No haematological changes were observed.

Key words: musk carrageenin oedema adrenalectomy phenylbutazone mycobacterium adjuvant histamine 5-HT

INTRODUCTION

Musk is the dried secretion from the prepucial follicles of musk deer, *Moschus moschiferus* Linn. Practitioners of the indigenous system of medicine have claimed beneficial results with musk in the treatment of arthritis and inflammatory conditions. Mishra *et al.* (9) studied the effect of musk on grannuloma formation in rats and found it to be an anti-inflammatory agent like hydrocortisone. Siddiqui (13) reported the anti-arthritic activity of musk in adjuvant arthritis in rats and found it to be more potent than phenylbutazone in inhibiting the primary phase of arthritis. In the present study, a detailed investigation of the anti-inflammatory activity of musk has been carried out in both acute and chronic models of inflammation and the activity has been compared with standard drugs like hydrocortisone, a steroidal drug and phenylbutazone, a non-steroidal drug. The investigation also includes studies on its possible mechanism of action.

MATERIALS AND METHODS

Musk samples were obtained through the courtesy of the Director, Central Council for for Research in Indian Medicine and Homoepathy, New Delhi. Since musk is liable to gross adulteration, the sample used for the study was tested for its authenticity. Upon the addition of a few grains of musk to 2 *ml* of chloroform in a watch glass, the grains float on the surface. When stirred with a glass rod, the solution remained nearly colourless. However, when it was evaporated, a small quantity of a whitish oil or fatty substance separated around the particles (3).

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For anti-inflammatory studies, musk was used as a suspension in Tween 80 (itself inactive) diluted with distilled water.

Carrageenin-induced oedema: Inflammation was produced in male albino rats weighing 100-200 *g* according to the method described by Winter *et al.* (17). Carrageenin was made into 1% suspension in normal saline and 0.05 *ml* was injected into the right hind foot of each rat under the planter aponeurosis. Treatments were administered one hr before the njection of carrageenin into the paw. The volume of the foot was measured before and 3 hr after the carrageenin injection by mercury displacement volume plethysmometer, modified by Harris and Spencer (5). Animals of different groups received hydrocortisone acetate or phenylbutazone or various doses of musk. Percentage inhibition of the swelling was calculated according to Newbould (10) as follows: Percentage inhibition = 100 (1-(a-x)/(b-y), y, was the mean foot thickness of control rats before injection b, was the mean foot thickness of control rats after the injection; x, was the mean foot thickness of treated rats before injection and a, the mean foot, thickness of treated rats after the injection. The formula was applied to all the methods emplyed in this study.

Formaline arthritis method : The method followed was that of Selye (12) as modified by Browallee (2).

Initial volume of the hind paw of each rat was measured by the mercury displacement volume plethysmometer. Formaline (0.1 *ml* of 2% solution) was injected into the hind foot of each rat under the planter aponeurosis on the first and the third day of experiment. Animals were pre-treated with test substances for one day before starting the experiment. The treatments were then administreed once daily for ten days. Measurement of foot volume was recorded daily for ten consequtive days. All the experiments were conducted under similar conditions of temperature in the following groups.

Adjuvant arthritis: Arthritis-like syndrome was produced by the method described by Newbould (10). An intradermal injection of 0.05 *ml* of fine suspension of dead tubercle bacilli inliquid paraffin (5 *mg/ml*) was given into the right hind paw of each rat under the planter spone-uresis. The tubercle bacilli were derived from the human strains PN, DT and C. The test substances were administered once daily for feurteen days, starting one day prior to the injection of the adjuvant. Foot measurements were made daily by the plethysmometer. Percentage inhibition of the inflammatory response was recorded on day thirteen of the experiment. Weight change of the animal from initial to the thirteenth day and the severity of development of secondary lesions were also observed.

Survival-time in adrenalectomized rats: Male albino rats weighing 80-100 *g* were divided into three groups of ten rats each. Bilateral adrenalectomy was performed according to the method of Schultzer (11). (Table II)

Haemotological studies: Effect of musk was studied on total white blood cells, lymphocyte and eosinophil count in non-adrenalectomized and adrenalectomized rats. Haematological

studies were performed five hr after the administration of test substances. (Table III)

Effect on histamine and 5-hydroxytryptamine (5-HT) content of rat paw skin: Effect of musk was studied on histamine and 5-HT content of rat paw skin in carrageenin inflammation. Thirty min after the injection of carrageenin, the animals were sacrificed. Paws of each rat were then severed just above the ankle joints. Skin of each paw was removed and extracted for histamine and 5-HT content. Details of different treatments are given in Table III.

Histamine estimation: The method followed for extraction was that of Feldberg and Talesnik (4). The extracts were assayed biologically against standard histamine acid phosphate on guinea-pig ileum suspended in a 15 *ml* organ bath cantaining 0.1 $\mu g/ml$ of atropine sulphate in oxygenated Tyrode solution at 37°C.

5-HT estimation: Extraction of 5-HT from the paw skin was done according to the method described by Amin et *al.* (1). The extract was assayed on isolated stomach stip of rat according to the method of Vane (14) as modified by Lin and Yooh (7) against standard 5-HT creatinine-complex in a 15 *ml* organ bath at 37.5°C.

Acute toxicity: The LD (50) of musk was determined in mice by intraperitoneal route by the method of Litchfield and Wilcoxon (8).

RESULTS

Carrageenin Oedema: Musk produced a marked inhibition of the carrageenin oedema. At a dose level of 0.5 *mg*/100 *g*, it produced 43.6% inhibition. Hydrocortisone at the same dose (Table I) produced less inhibition. Musk (1 *mg*/100 *g*) produced 59% inhibition which was slightly less than those produced by 10 *mg*/100 *g* phenylbutazone. The differences were highly significant ($P<0.001$) and the effects dose related.

Formaline arthritis: Following an injection of 0.1 *ml* of 2 percent formaldehyde solution acute oedema of the paw occurred in all animals, which was less marked on the third day when a socond injection of formaldehyde was given. The swelling again started increasing till the fifth or the sixth day, when a chronic arthritis of the ankle joint was apparent, maximum swelling occurred in the ninth or tenth day. Musk (1 *mg*) produced 52.5% inhibition of the oedema ($P<0.001$). At a dose of 5 *mg* it was nore potent than phenylbutazone, producing an inhibition of 56.1 percent (Table I).

Adjuvant arthritis: The results are given in Table I. Injection of 0.05 *ml* of a fine suspension of Freund's adjuvant into the rat paw produced a severe arthritis of the ankle joints which reached its maximum on the fourth day, slowly subsiding till eighth day, after which it again started increasing in size till it reached its maximum on the thirteenth day. Secondary lessions (inflammation of the other paws, ears and tail) started appearing on the tenth or eleventh day. Musk, in all the doses used, was highly offective in inhibiting the full development of the primary lession in the adjuvant arthritis. At a dose of 1 *mg*/100 g it produced 60.5

percent inhibition and at 5 *mg* it produced 72.3 percent inhibition. It was more pote[illegible] than phenylbutazone and hydrocortisone.

TABLE I: Effect of musk, hydrocortisone and phenylbutazone on experimental inflammatory responses in r[illegible]

Type of inflammation	*Treatment (mg/100 g)*	*Mean % inhibition of oedema ± S.E.*	*Mean weight loss ± S.E.*	*Secon[illegible] lesion*
Carrageenin oedema	1. Control (12)	—		
	2. Hydrocortisone acetate, 0.5, ip (12)	37.25 ± 5.3*		
	3. Phenylbutazone 10.0, orally (11)	66.66 ± 4.26**		
	4. Musk, 0.5, ip (12)	43.6 ± 5.17*		
	5. Musk, 1.0, ip (12)	59.0 ± 4.5**		
	6. Musk, 5.0, ip (12)	70.8 ± 2.6**		
Formaline arthritis	1. Control (12)	—		
	2. Hydrocortisone acetate, 0.5, ip (12)	35.59 ± 3.56**		
	3. Phonylbutazone, 10.0, orally (11)	62.7 ± 4·23**		
	4. Musk, 1.0, ip (12)	52.5 ± 5.10**		
	5. Musk, 5.0, ip (12)	66.1 ± 4.83**		
Adjuvant arthritis	1. Control (12)	—	—55.5 ± 9.2	Severe
	2. Hydrocortisone acetate, 0.5, ip (11)	38.1 ± 2.8**	—52 ± 8.6	Moderate
	3. Phenylbutazone, 10.0, orally (11)	62.6 ± 3.6**	—14.5 ± 3.2	Mild
	4. Musk, 1.0, ip (12)	60.5 ± 4.2**	—13 ± 4.8	Mlid
	5. Musk, 5.0, ip (12)	72.3 ± 4.8**	—14.5 ± 4.04	Mild

Figures in parentheses indicate the number of animals. Significance of difference between control and expe[illegible] mental groups has been calculated by Students 't' test. *P<0.01, ** P<0.001.

Secondary lesions were mild with phenylbutazone and musk, moderate with hydrocortison[illegible] acetate and severe in the control group. The time of appearance of secondary lesions in all th[illegible] groups including the control was about the same, i.e., tenth or eleventh day of the experiment[illegible] Weight loss with phenylbutazone and musk aws almost the same (with musk, -13 *g* and with[illegible] phenylbutazone, -14.5 *g*). With hydrocortisone acetate, the weight loss was the same as tha[illegible] of the control group (-52 *g*).

Survival time after adrenalectomy: The mean survival time of adremalectonized rat[illegible] was 23.4 hr. Dextrose saline significantly prolonged the mean survival period of adrenalectomiz[illegible]ed rats to 66.5 hr (P<0.001). The effect of dextrose saline was not enhanced by the addition[illegible] of musk (Table II) indicating that the latter had no action of its own in this respect.

TABLE II: Effect of musk on survival-time of adrenal-ectomized rats.

Treatment	*Mean survival time (hr) ± S.E.*
Control (10)	23.4 ± 0.82
Dextrose-saline, 2.5 *ml*, sc (10)	66.5 ± 1.20*
Dextrose-saline, 2.5 *ml*, sc and Musk 1 *mg*/100 g ip (10)	67.4 ± 1.40

Figures in parentheses indicate the numbers of animals. Dextrose—saline consisted of 5% dextrose and 1% sodium chloride. Effect of musk on survival time has been compared with dextrose—saline as the control. The significance of difference has been calculated by Students 't' test. *P<0.001.

Effect on haematological parameters: Musk at a dose of 1 *mg*/100 *g* did not affect the total white blood, cell, lymphocyte and cosinophil counts after five hr of the treatment, both in normal and adrenalectomized rats (Table III).

TABLE III: Effect of musk on haematological parameters.

Group	*Treatment*	*Mean total white cell count per cmm blood ± S.E.*	*Mean total lymphocyte count per cmm blood ± S.E.*	*Mean total cesinophil count per cmm blood ± S.E.*
Non-adrenalectomized rats:	Control (10)	10693 ± 465.8	6675 ± 317.4	601 ± 33.8
	Musk (10) 1 *mg*/100 g ip	10914 ± 410.4	6766 ± 226.8.	582 ± 22.2
Adrenalectomized rats :	Control (10)	10059 ± 333.8	5869 ± 426.5	526 ± 21.8
	Musk (10) 1 *mg*/100 g ip	10340 ± 157.2	6077 ± 166.4	498 ± 20.8

Figures in parentheses indicate number of animals.

Effect on histamine and 5-HT contents: The histamine content in normal rat paw skin was 29.19 $\mu g/g$ tissue. The histamine content of skin when estimated thirty min after the injection of a carrageenin increased to 84.93 $\mu g/g$ of tissue. Animals pretreated with 1 *mg*/100 *g* of musk ip showed marked decline in the histamine content was 5.51 $\mu g/g$ of tissue in the group treated with musk. The reduction in histamine content by musk was highly significant ($P<0.001$), (Table IV).

TABLE IV: Effect of musk on histamine and and 5-HT content of rat paw skin.

Treatment	*Mean histamine content $\mu g/g$ ± S.E.*	*Mean 5-HT content $\mu g/g$ ± S.E.*
Normal	29.19 ± 3.3 (8)	75.5 ± 1.5 (6)
Inflammed (carrageenin-induced)	84.93 ± 7.2* (8)	71.5 ± 6.0 (6)
Musk 1 *mg*/100 *g* ip	5.81 ± 1.29 (8)	11.2 ± 0.7*(6)

Figures in parentheses indicate the number of observations. The significance of difference between normal and expeirmental group has been calculated by Students 't' test. * P<0.001.

The 5-HT content of normal rat paw skin was 75.5 *ng/g* of tissue. No increa 5-HT content occured after carrageenin injection in the rat foot. Animals pretreated 1 *mg*/100 *g* of musk showed marked decrease in 5-HT content. The 5-HT content of rat skin was 11.2 *ng/g* of tissue in musk treated group. The difference between the two g was highly significant ($P<0.001$). (Table IV).

Acute texicity: The ip LD 50 of musk in mice was 310 *mg/kg*.

DISCUSSION

The anti-inflammatory activity of Musk has been tested using both immunological non-immunological methods. Each mothod represents a different model of inflammation.

Musk has been shown to be very effective in reducing the carrageening-induced oed Carrageenin-induced oedema represents an acute oxudative form of inflammation. It has demonstrated by Leme et *al.* (6), that the mediators involved in the production of carrag oedema including a variety of macromolecular compounds. They showed that histamine 5-HT do not play a part in the development of carrageenin oedema since anti-histamine or 5-HT agents had no influence on the development of oedema. In the present investigati although there was no increase in the 5-HT content of rat paw skin after carrageenin injec there was a marked and significant increase in the histamine content. Histamine and 5 contents were measured 30 min after carrageenin injection, on the basis of the observation Vinegar *et al.* (15), who showed that carrageenin oedema develops as biphasic event, an in phase lasting for 30 min to 1 hr and a secondary phase lasting from 2 to 3 hr. Our result dicate that histamine plays an important role in the development of early phase of carrage oedema. The results are also supported by the finding that musk markedly lowered the hista content of the skin. As far as the 5-HT content is concerned the results are similar to those Leme *et al.* (6). Leme *et al.* (6) suggested non-involvement of histamine as a mediator carrageenin oedema, however, they did not extract and estimate histamine during the p of the early phase of carrageenin oedema. Our findings also indicate that 5-HT plays no r in the early phase of carrageenin oedema. Musk lowered the normal 5-HT content of the tis Our observations suggest that in the first phase of carrageenin oedema, histamine may be ing as a chief mediator and that the beneficial effect of musk may be due to decrease in histamine content of the rat paw.

Formaline arthritis represents a chronicprroliferative type of inflammation. Musk h marked inhibitory effect in this type of arthritis.

Adjuvant arthritis represents an immunological model of inflammation (16). The arthr induced by Freund's adjuvant has aroused wide biological interest since this experimental c dition elicits an immunological process comparable in some aspects to that probably underly the rheumatic and arthritic syndrome in man. Musk effectively inhibited the full developmen

polyarthritis, induced by Feund's adjuvant. Secondary lesions observed were only mild and tl.e weight loss was minimal.

Our experiments indicate that musk is a very effective anti-inflammatory agent in both acute and chronic models of inflammation in animals. Its anti-inflammatory activity may be attributed to the reduction in the histamine and 5-HT contents of the inflammed tissues.

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