
Studies on *Ocimum gratissimum* Seed Mucilage: Evaluation of Suspending Properties

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Mucilage extracted from *Ocimum gratissimum* seeds were subjected to toxicity studies for its safety and preformulation studies for its suitability as a suspending agent. Zinc oxide suspensions were prepared and compared with different concentrations of *Ocimum gratissimum* mucilage, tragacanth and sodium CMC. The mucilage extracted is devoid of toxicity. The mucilage was found to be a superior suspending agent than tragacanth and is comparable to sodium CMC. Studies indicate that the extracted mucilage may be a good source as a pharmaceutical adjuvant specifically as a suspending agent.

Mucilages and gums are well known since ancient times for their medicinal use. In modern era also they are widely used in the pharmaceutical industries as thickeners, water retention agents, emulsion stabilizers, suspending agents, binders and film formers^{1,2}. Apart from its use in the finished medicines, newer uses have been found in cosmetics, textiles and paint paper³. Naturally demand for these substances is increasing and new sources are getting tapped^{4,5}. India due to geographical and environmental positioning has traditionally been a good source for such products among the Asian countries. But still a large quantity of this is being imported from the European countries to meet up the everdemand⁶.

Ocimum gratissimum Linn. Var. cinerifolium (Benz), fam: Labiatae, is a semi woody tender perennial 3 to 8 feet in height, which is grown widely in India, because of its social and religious importance. The plant oil is an established source of mosquito repellent, antibacterial and local anaesthetic¹⁰. However, no work has been reported so far the usage of the gums extracted from the seed. The present work is an attempt to extract and investigate the pharmaceutical properties of the gum to assess its suitability as a suspending agent in the pharmaceutical formulation.

MATERIALS AND METHODS

Zinc oxide (E. Merck, Mumbai), Tragacanth (CDH, Mumbai), Sodium carboxy methylcellulose (Loba Chemie, Mumbai) was procured from the open market. All the solvents and reagents used were of analytical grade. *Ocimum* seeds were obtained from the medicinal garden, Birla Institute of Technology, Mesra, Ranchi.

Isolation of mucilage:

The cleaned seeds were defatted with petroleum ether (60-80) in a Soxhlet. The defatted material (25 g) was soaked in distilled water (500 ml) at room temperature for 12 h. The resulting mass was stirred at about 100 rpm for 1 h and strained through muslin cloth. To the filtrate, acetone was added until precipitation was complete. The precipitated mucilage was filtered through muslin cloth and the mucilaginous residue was spread on glass plates and dried at 40°. The extracted polysaccharide (4 g) was dispersed in 200 ml water with stirring for 12 h and ethanol was added in different proportion. First the concentration of ethanol was made up to 20% in the solution. Some impurities that precipitated were removed by centrifugation. The ethanol concentration was further increased to 60% to precipitate the remaining polysaccharides. The precipitated gum was filtered, treated with acetone to remove the traces of water and dried¹¹ in an oven at 40°.

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Evaluation of toxicity:

A toxicity study was carried out according to Knudsen and Curtis¹². The animals used in the toxicity studies were sanctioned by the Institute Animal Ethics Committee (approved by CPCSEA, Chennai, India). Swiss mice weighing 20-25 g of either sex aged 8-10 w were fasted for 18 h and used. In the acute toxicity studies a dose of 250 mg/kg was administered orally to twelve mice with an additional six kept as control. Then they were observed for motor reflexes for 48 h. Since no mortality was observed and behavioral pattern was un affected, further studies were carried out using a different set of animals (12 test and 6 control) where a dose of 500 mg/kg was administered and the animals were observed for 72 h. No toxic manifestations were observed. In the second phase of chronic toxicity studies, 22 animals were used, divided into two groups, 6 as control and 16 as test animals. In the test group a dose of 250 mg/kg was administered daily for a period of 30 d. Body weights were recorded for both the groups at an interval of 10 d. And at the end, hematological parameters were studied in both the groups.

Preparation of zinc oxide suspension:

Zinc oxide which was sieved through sieve No. 100 and retained on sieve No. 200 was used to yield a 20% w/v suspension in water using tragacanth, sodium CMC and ocimum mucilage. Using each suspending agents suspensions were produced with four concentrations (0.5, 1.0, 1.5 and 2.0% w/v). For making the suspensions, zinc oxide was first levigated with glycerin (1:1). Then a weighed amount of these suspending agents were added and triturated and finally the volume was made up with distilled water. The suspension contained 0.1% w/v benzoic acid as a preservative. All the suspensions were deflocculated. To determine the degree of flocculation we have also prepared flocculated suspension using a flocculating agent, potassium dihydrogenphosphate (0.004 mol).

Rate of separation:

The rate of separation of the suspensions were studied by keeping 50 ml portion of each suspensions in a stoppered measuring cylinder and stored undisturbed at room temperature. The separation of clear liquid was noted at intervals of 5 d up to 45 d. The separation ratio was calculated using the formula H_s/H_o , where H_s is the height of clear liquid and H_o is the original height of the sample¹³.

Degree of flocculation:

The degree of flocculation¹⁴ was determined from the

following equation $\beta = F/F\alpha$, where F is the ultimate sedimentation volume in the flocculated suspension and $F\alpha$ is the ultimate sedimentation volume in the deflocculated suspension.

Redispersion:

Fixed volume (50 ml) of the each suspension was kept in calibrated tubes which were then stored at room temperature for various time intervals (5, 10.... 45 d). At regular interval of 5 d, one tube was removed and shaken vigorously to redistribute the sediment and the presence of deposit if any is recorded.

RESULTS AND DISCUSSION

The average yield of dried mucilage obtained from ocimum seed was 13.5 %. To determine the safety level of the extracted ocimum mucilage, acute as well as chronic toxicity studies were carried out. In both the studies no manifestation of toxic syndromes were observed. To assess the suitability of gum for the oral delivery we have recorded the body weight profile for the animals during the chronic toxicity studies at regular intervals of 10 d. It was found that the body weight of both test and control and the rate of increase were also comparable. Hence it can be inferred that chronic administration of the gum might not influence either the food intake or growth. Hematological parameters that were determined at the end of 30 d of continuous administration were also found to be comparable to that of normal mice (Table 1). The effective concentration of the suspending agent in the conventional dosage form normally does not exceed 2% of the formulation, which is approximately 5-10 mg/kg dose. Hence this excipient is not likely to exert any toxic effect on the body.

TABLE 1: EFFECT OF OCIMUM MUCILAGE ON MICE¹

Hematological parameters	Ocimum treated ²	Control ²
Bleeding time in minutes	4.15±0.09	4.18±0.07
Clotting time in seconds	39.0±0.00	36.0±0.06
Total Count of RBC/mm ³	9.61×10 ⁶ ±0.75	9.93×10 ⁶ ±0.02
Total Count of WBC/mm ³	4250±0.17	4350±0.13
Hemoglobin Content	14.0±0.06	14.1±0.00

¹Data was recorded after 30 d of chronic oral administration of ocimum mucilage. ²Data represents as the mean±SD of six readings.

It is quite well understood that the better is the suspending medium the lesser the rate of sedimentation¹⁵. Suspensions are routinely evaluated for their rate of separation which indicates its suspending property. To evaluate the suspending properties of the gum, suspensions were prepared with fixed concentration¹⁶ of zinc oxide (20% w/v) but with varying concentration of test mucilage (0.5 to 2.5% w/v) as well as the traditional suspending agents such as tragacanth and sodium CMC¹⁷. Fig.1 shows a comparison of the efficacy of suspending agents when used in low concentration (0.5% w/v). Here ocimum mucilage shows its superiority over tragacanth. In fig. 2 where comparison was done at 1% w/v level of suspending agents, ocimum showed a comparable result to that of sodium CMC. As expected the sedimentation rate was much lower to that of the 0.5% w/v level. At 1.5% and 2% w/v concentration, ocimum mucilage had shown better results than its counterparts.

According to Martin *et al.*¹⁸ the sedimentation volume provides only a qualitative account of flocculation. The degree of flocculation (β) is a more useful parameter. This is the ratio of the ultimate sediment volume in the flocculated and deflocculated systems. A comparison of the β value

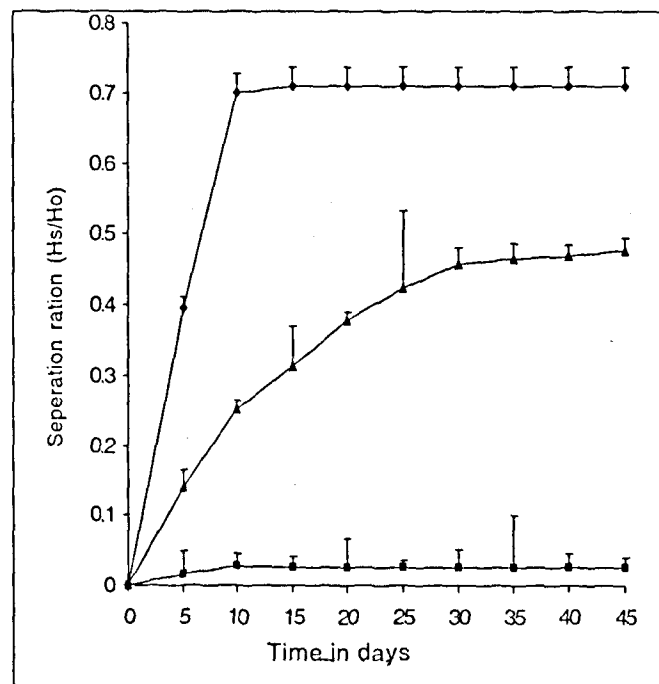


Fig. 1: comparison of suspending properties at 0.5 % level

Sedimentation behaviour of zinc oxide suspensions of (-♦-) tragacanth, (-■-) sodium CMC and (-▲-) ocimum mucilage at 0.5% w/v concentration.

(Table 2) of suspensions prepared with tragacanth, sodium CMC and ocimum mucilage shows higher values at the 1.5 and 2% w/v level for ocimum and sodium CMC. These observations show that ocimum is a better suspending agent than tragacanth.

Since the suspension produces sediment on storage, it must be readily dispersible so as to ensure the uniformity of the dose. If after shaking vigorously for specified time and the deposit is still present, the system is described¹⁹ as caked. The suspensions with 0.5% and 1.0% w/v tragacanth have shown to be caked after 30 and 40 d, respectively, indicating its ineffectiveness as suspending agent at this concentration. However, the suspensions with sodium CMC and Ocimum mucilage were easily redispersible, irrespective of their concentrations. Thus it can be concluded that the extracted mucilage from seeds of *Ocimum gratissimum* is non-toxic, has the potential as a suspending agent even at low concentration and can be used as a pharmaceutical adjuvant.

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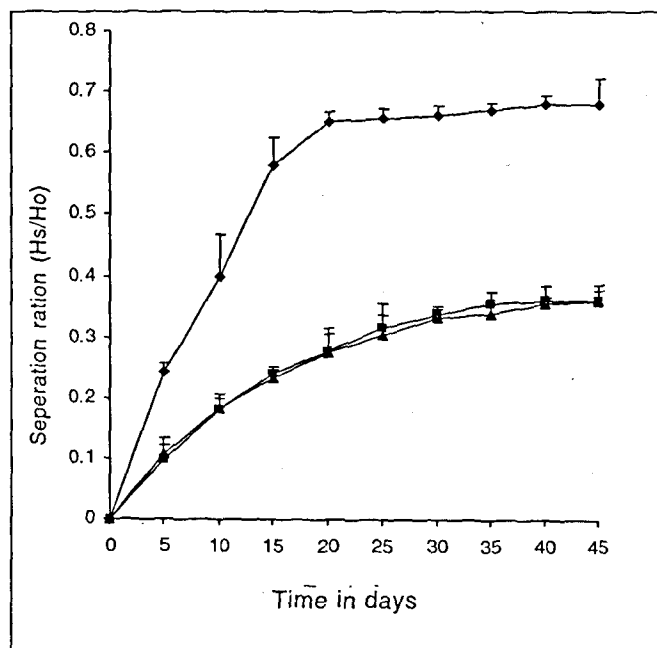


Fig. 2: comparison of suspending properties at 1 % level

Sedimentation behaviour of zinc oxide suspensions of (-♦-) tragacanth, (-■-) sodium CMC and (-▲-) ocimum mucilage at 1% w/v concentration.

TABLE 2: DEGREE OF FLOCCULATION (β) BOF VARIOUS SUSPENDING AGENTS¹.

Suspending agent	Concentration (% w/v)	Degree of Flocculation (β) ²
Tragacanth	0.5	-
	1.0	-
	1.5	1.82±0.00
	2.0	2.64±0.09
Sodium CMC	0.5	2.38±0.06
	1.0	2.89±0.11
	1.5	3.54±0.15
	2.0	3.89±0.10
Ocimum Seed Mucilage	0.5	2.36±0.00
	1.0	2.91±0.00
	1.5	3.58±0.01
	2.0	3.93±0.01

¹Data was recorded after 45 d keeping at room temperature (25°). ²Data represents as the mean±SD of three readings.

- Suspensions were found to be caked.

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