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LETTER TO THE EDITOR

STUDY OF ANTI-TUSSIVE ACTIVITY OF *OCIMUM SANCTUM* LINN IN GUINEA PIGS

Sir,

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Ocimum sanctum Linn (Labiatae) popularly known as the holy basil or Tulsi in India is a home remedy for various illnesses. Traditionally, the fresh fruit and leaf juice were commonly used in the treatment of cough as demulcent, mild upper respiratory tract infection, general stress syndrome, worm infestations, superficial fungal infections, and also as a diuretic (1). This plant has been evaluated pharmacologically for immunomodulatory, antistress, antimicrobial, anti-inflammatory antiasthmatic, hypoglycemic, hypotensive and analgesic activities and found to be effective in varying degrees in the animal models (2). The plant has also shown significant anti-oxidant activity (3). *O. sanctum* is reported to be well tolerated upto a dose of 5–7 g/day for 3 months except for constipation in few cases (4). The crude forms of the plant and the extracts are used singularly or in combination with other herbs as a cough remedy and expectorant based on the traditional experience. However the role of *Ocimum sanctum* as an antitussive has not been scientifically evaluated. The present study was therefore conducted to validate the traditional claims of this plant.

The whole plant of *Ocimum sanctum* L. was collected in the month of August 2003 from Bangalore. It was shade dried, powdered and stored in a clean area free from pests. The sample was authenticated by NISCAIR, New Delhi and the voucher specimen (RHM/F-3/2001/298) was deposited with department of Pharmocognosy, Natural Remedies Pvt. Ltd., Bangalore.

Aqueous and methonolic extract of *Ocimum sanctum* were prepared from air dried, powdered aerial parts of the plant. The plant material was extracted by hot percolations and dried under vacuum to remove traces of the solvent. The yields were 18% w/w and 6.1% w/w for water and methanolic extracts respectively. The HPTLC analysis revealed the presence of ursolic acid 1.3% in methanolic and 0.5% in aqueous extracts The chromatogram of both the extracts had similar peaks by HPLC method.

Healthy male guinea pigs (500 ± 150 g) were maintained at 25–27° C room temperature with free access to laboratory guinea pig pellets (supplemented with greens) and filtered tap water. The day before the test, they were placed individually in histamine chamber for 5 minutes for acclimatization.

Cough was induced by exposure to the aerosol of citric acid (7.5% w/v) introduced through a small opening at the sides of the chamber using an ultrasonic nebulizer (Microlux, Italy) for 5 minutes. The frequency of cough during following 5 minutes was recorded. The animals showing 15 ± 5 bouts of cough were selected for the study (5).

The selected animals were randomly assigned to 3 groups with six animals in each group. After an hour of rest the animals were administered codeine phosphate 25 mg,

aqueous extract 1.55 gms and methanolic extract 0.875 gms/kg body wt respectively. The drugs were administered orally. An hour after drug administration, they were subjected to citric acid aerosol exposure again and the number of bouts were recorded as mentioned above.

The number of bouts of cough in 5 minutes expressed as mean ± SEM before and after treatment in each group using students' paired 't' test. $P<0.05$ was considered statistically significant.

Out of 24 animals screened for aerosol testing, only 18 were found to exhibit a cough response with the frequency of 15 ± 5 and hence qualified for the test. The standard drug codeine phosphate brought about a reduction of bouts of cough from 16.17 ± 1.30 to 0.33 ± 0.33 (98.41% inhibition), which was significant ($P<.001$). Aqueous extract at dose of 1.55 gms per kg body wt. showed a reduction from 17.17 ± 1.17 to 5.17 ± 2.14 (72.5% Inhibition, $P<0.05$) and methanolic extract at the dose of 875 mgs per kg body wt. showed a reduction from 15.50 ± 1.54 to 9.83 ± 1.14 (35.39% inhibition, $P<0.05$) (Table I).

The dose of aqueous extract was extrapolated from human dose of 2 gms as suggested by Ayurvedic texts (1, 6). The Ursolic acid content in aqueous extract was 0.5% while that of methanolic extract was 1.3%. Ursolic acid has been identified to be an active principle for antioxidant activity (7) and therefore a dose of methanolic extract that matched with the aqueous extract in its content of ursolic acid was chosen. Codeine phosphate was chosen as the standard drug at a dose of 25 mg/kg body weight. The study showed that both the test extracts posses significant antitussive activity and AE showed a higher activity than the ME. Therefore it appears that there could be active principles in addition to ursolic acid responsible for antitussive activity.

Citric acid induced cough model in conscious guinea pigs has been used as a sensitive model for evaluation of some of the centrally acting opioid antitussives like codeine and morphine (8). Evidences from recent studies on cough induced by capsaicin and citric acid and its reversal by capsazepin (cap receptor antagonist) indicate that the effects of citric acid are mediated through cap receptor. Citric acid can produce its effect on cough receptors either by direct action or by the production of an intermediate agent released by irritation of mucosa (9). Narcotic analgesics bring about cough suppression in this model by stimulating the opioid receptors in the cough center (10). In addition, the possible involvement of GABA-ergic and serotonergic mechanisms have also been implicated in the anti-tussive action of these drugs (11).

Previous studies on *Ocimum sanctum* have demonstrated that ethanolic extract of leaves of this plant possess anti-stress

TABLE I: Effect of *Ocimum sanctum* on citric acid evoked cough in guinea pigs.

Sl. No.	*Group*	*Dose gms/kg*	*n*	*Frequency of cough %*		
				Before	*After*	*Inhibition*
1.	Codeine Linctus	0.25	6	16.17±1.30	0.33±0.33[a]	98.41±1.59
2.	O. sanctum aqueous extract	1.55	6	17.17±1.17	5.17±2.41[b]	72.5±12.8
3.	O. sanctum methanolic extract	0.875	6	15.50±1.54	9.83±1.14[c]	35.39±7.55

N=Number of animals per group; Values are Mean ± SEM; [a]$P\leq0.001$; [b]$P\leq0.01$; [a]$P\leq0.05$.

activity. The ethanolic extract at a dose of 400 mgs/kg intraperitoneally increased the swimming time in albino rats (12). The extract also decreased the apomorphine induced fighting time and ambulation in open field studies thus establishing the anti-anxiety effect (13).

The above activities clearly indicate that the plant has significant effect on the central nervous system bringing about antistress and anxiolytic effect that may involve the GABA-ergic system.

Khanna and Bhatia have also demonstrated the antinociceptive activity of *O. Sanctum.* The alcoholic extract increased the tail-flick withdrawal latency in mice which was reversed with naloxone indicating the involvement of opioid receptors in the analgesic activity (14).

Based on this review it appears that *O. Sanctum* brings about its antitussive effect by central action probably mediated by both opioid system & GABA-ergic system. Confirmatory studies using specific antagonists in the same experimental model would establish this mechanism conclusively.

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