

## Antiemetic and Anti-inflammatory activity of leaves and flower extracts of *Luffa cylindrica* (L.) Roem

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**Abstract**

Ethanol and hexane extracts of the leaves and male flowers of *Luffa cylindrica* were evaluated for

### 1. Introduction

*Luffa cylindrica* (family Cucurbitaceae) is an annual climbing or trailing herb which is cultivated in Pakistan at Jehlum, Jammu and Kashmir, Loralai and Karachi (Nazimuddin, 1984). The plant is reported as laxative and useful in asthma, intestinal worms, sinusitis (Chakravarty, 1990; Schultes, 1990), oedema, mastitis, pharyngitis and rhinitis (Khare, 2007). Leaves are used in decayed teeth, parasitic affections, skin diseases (Porterfield, 1955), chronic bronchitis (Khare, 2007) and reported as an emmenagogue and diuretic (Perry, 1980). Seeds of *L. cylindrica* are carthartic (Prajapati et al., 2003). The stem is used in respiratory complaints. Riped fruits are reported as antiseptic, anthelmintic, carminative, emmenagogue, galactagogue, tonic to the genital organs, used in the

antiemetic and anti-inflammatory effect using chick emesis model and carrageenan induced rat paw oedema. The antiemetic effect was observed at a dose of 150 mg/kg body weight whereas anti-inflammatory effect was observed at doses of 500, 750 and 1000 mg/kg body weight orally. Chlorpromazine 150 mg/kg and indomethacin 10 mg/kg orally were used as standard antiemetic and anti-inflammatory drugs. The antiemetic effect was determined by calculating the mean decrease in number of retching in contrast with those of control after 10 minutes of copper sulfate (50 mg/kg orally) administration. The degree of paw oedema of all the groups was measured using a plethysmometer at 5<sup>th</sup> hour of carrageenan (1% w/v) administration. All extracts except hexane extract of leaves exhibited statistically significant ( $P<0.001$ ) antiemetic and except hexane extract of flower, all extracts exhibited statistically significant ( $P<0.05$ ) antiinflammatory effects.

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treatment of hernia, hemorrhoids, jaundice, menorrhagia, scarlet fever (Porterfield, 1955), bronchitis, haematuria, leprosy, spleenopathy, syphilis (Prajapati et al., 2003). Crushed leaves are used in various states of pain and inflammation as in carbuncles, abcesses, swellings and heat rashes of children in summer (Perry, 1980) and crushed flowers are used for treating migraine (Khare, 2007). Phytochemical investigation of *Luffa cylindrica* reveals that leaves contain flavonoids (Schilling and Heiser, 1981), saponins (Liang et al., 1993 and 1996) and triterpenes (Nauking Institute of Materia Medica, 1980), while flowers contain flavonoids (Schilling and Heiser, 1981). The fruit contains triterpenoid saponins and seed contains polypeptides (Partap et al., 2012). Whole plant reported to

possess anti-tussive, anti-asthmatic, cardiac stimulant, hepatoprotective, hypolipidemic whereas fruits, leaves and stem extracts possess immunostimulant activity (Partap et al., 2012).

Copper sulphate induced chick emesis model is frequently used for evaluating natural antiemetics (Ahmed et al., 2012a,b ; Hasan et al., 2012a,b; Quds et al., 2012) due to its easiness, simplicity and authenticity (Akita et al., 1998). This is the first report to evaluate the antiemetic activity of *Luffa cylindrica*. Anti-inflammatory activity of *Luffa cylindrica* plant (Abirami et al., 2011) and its seeds (Muthumani et al., 2010) using carrageenan induced rat paw edema has been published earlier but there is no published report regarding the anti-inflammatory activity of leaves and flowers. Present study declares the anti-inflammatory activity of hexane and ethanol extracts of the leaves and male flowers of *Luffa cylindrica* (L.) Roem. The antiemetic effect of *Luffa cylindrica* is reported for the first time.

## 2. Materials and methods

### 2.1 Plant material collection, identification and crude extract preparation

The leaves and male flowers were collected from Malir, Karachi, Pakistan in June 2011. The plant material was identified by a taxonomist and voucher specimen (G.H.No.85993) was deposited in the herbarium of Department of Botany, University of Karachi. Leaves and flowers were soaked in ethanol and hexane separately for one week. All the extracts were filtered and concentrated to dryness in vacuum at 40°C by rotary evaporator.

### 2.2 Animals

Thirty six chicks in six different groups (N=6 for each group) were used for the antiemetic study. Young male chicks, 4 days of age, weighing from 32-52 g were taken from local market. After 24 hrs fasting, the antiemetic activity was evaluated. The first group (Group I) represented control group which received 1% tween 80 in water, orally at a dose of 10 ml/kg b.w., and the second group (Group II) received standard antiemetic drug Chlorpromazine (150 mg/kg orally). Group III, IV- received ethanol and hexane extract of leaves and Group V, VI- received ethanol and hexane extract of flowers (150mg/kg b.w., orally) respectively.

For anti-inflammatory activity, male Whistar Albino rats (weighing 150-200 g), were

procured from animal house of Aga Khan University and Hospital, Karachi. The animals were grouped with not more than six animals per cage. All tested extracts were used at doses of 500, 750 and 1000 mg/kg b.w., orally. Group No. I represented 1% tween 80 in water, orally at a dose of 10 ml/kg b.w., as control. Group II represented Indomethacin (10 mg/kg orally) as standard anti-inflammatory drug. Group III, IV,V and VI, VII,VIII showed ethanol and hexane extract of leaves respectively. Group IX, X, XI and XII,XIII,XIV received the ethanol and hexane extract of flowers.

The animals were maintained under standard laboratory conditions (temperature 25±2°C) and fed with standard pellet diet and fresh water ad libitum. All the animals were acclimatized to laboratory condition for a week before commencement of experiment. Permission and approval for animal studies were obtained from Board of Advanced Studies and Research, University of Karachi [BASR.Res.No.5(4)-2007].

### 2.3 Chemicals used

Copper (II) sulfate pentahydrate (copper sulfate) was purchased from Scharlau Chemie S.A. Barcelona, Spain. 3-(2-chloro-10H-phenothiazin-10-yl)-N,N-dimethyl-propan-1-amine (chlorpromazine) was purchased from ICN, USA. Dimethyl sulfoxide (DMSO), Polyoxyethylene sorbitan monooleate (Tween 80) and methanol were purchased from Merck, Darmstadt, Germany. 2-{1-[(4-chlorophenyl)carbonyl]-5-methoxy-2-methyl-1H-indol-3-yl}acetic acid (indomethacin) was purchased from Sigma-Aldrich Corporation.

### 2.4 Antiemetic activity

The antiemetic activity was determined by following the protocols of Akita et al., 1998. Each chick was set aside for 10 minutes to stabilize in a large beaker. Chlorpromazine and the extracts were dissolved in 0.9 % saline containing 5 % DMSO and 1 % tween 80 and administered abdominally at a dose of 150 mg/kg b.w., to the test animal. After 10 minutes copper sulfate was administered orally at 50 mg/kg b.w., to each chick, then the number of retching was observed during the next 10 minutes.

The percent inhibition was calculated by the following formula:

$$\text{Inhibition (\%)} = [(A-B)/A] \times 100$$

Where A = Frequency of retching in control group

B = Frequency of retching in test groups

### 2.5 Anti-inflammatory activity

The anti-inflammatory activity of the extracts was determined according to the method of Vogel and Vogel, (1997). All the suspensions were administered 30 min before the induction of oedema by administering 0.1 ml of 1% w/v carrageenan in saline. The degree of paw oedema of all the groups was measured using a plethysmometer at 5<sup>th</sup> hour of carrageenan administration to each group.

% Inhibition was calculated using the formula:

$$\% \text{ Inhibition (treated)} = \frac{V_5 - V_0}{V_0} \times 100$$

Where  $V_5$  and  $V_0$  represent Right hind paw volume at 5<sup>th</sup> hour after and before sub-plantar injection of carrageenan, respectively.

### 2.6 Statistical Analysis

All data were expressed as the mean  $\pm$  S. E. M. The data was analyzed by using unpaired Student's t-test and  $P < 0.001$  (for antiemetic activity)  $P < 0.05$  (for anti-inflammatory activity) were considered statistically significant.

## 3. Results

### 3.1 Antiemetic activity

The antiemetic effects of leaves and male flowers of *Luffa cylindrica* are shown in table-3.1.1. and present graphically in figure-3.1.2. Maximum antiemetic effect was observed by the hexane extract of flowers (71. 75 % inhibition of retches) whereas hexane extract of leaves showed minimum activity (43.5 % inhibition of retches). The ethanol extract of flowers and leaves showed 68.46 and 68.66 % inhibition of retches. The standard drug chlorpromazine showed 32.99 % inhibition of retches.

### 3.2 Anti-inflammatory activity

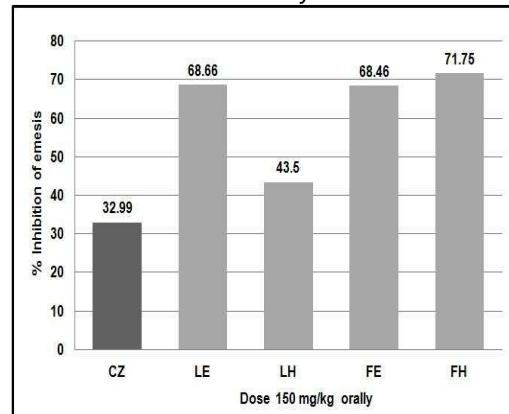
The data of anti-inflammatory activity is presented in table-3.2.1. The anti-inflammatory activity on carageenan induced rat paw oedema was compared to that of control on the basis of percent inhibition of paw oedema volume. The results showed that the extracts except hexane extract of flower exhibited statistically significant ( $P < 0.05$ ) inhibition of paw volume. Maximum percent inhibition of paw oedema in all the doses was found at 1000mg/kg b.w which is 68.28 (LH), 87.79 (LE) and 67.0 (FE) as shown in figure-3.2.2. Whereas hexane extract of flower in all three doses did not show anti-inflammatory effect. The standard drug indomethacin showed 66.59 % inhibition at dose of 10 mg/kg body weight.

**Table 3.1.1** The antiemetic effect of the leaves and flower extracts of *Luffa cylindrica* in chicks.

Drug (orally)	Number of Retches (Mean $\pm$ SEM)	% Inhibition of emesis
Control	69.28 $\pm$ 4.28	-
CZ	46.42 $\pm$ 4.25*	32.99
LE	21.71 $\pm$ 1.12*	68.66
LH	39.14 $\pm$ 0.66	43.5
FE	21.85 $\pm$ 1.27*	68.46
FH	19.57 $\pm$ 0.71*	71.75

CZ = Chlorpromazine, LE = Ethanol Extract of Leaves, LH = Hexane Extract of Leaves, FE = Ethanol Extract of Flowers, FH = Hexane Extract of Flowers, N=6, Dose=150 mg/kg orally. \* $P < 0.001$  is significantly different from control value using unpaired student's t-test.

**Figure 3.1.2:** The antiemetic effect of the leaves and flower extracts of *Luffa cylindrica* in chicks.



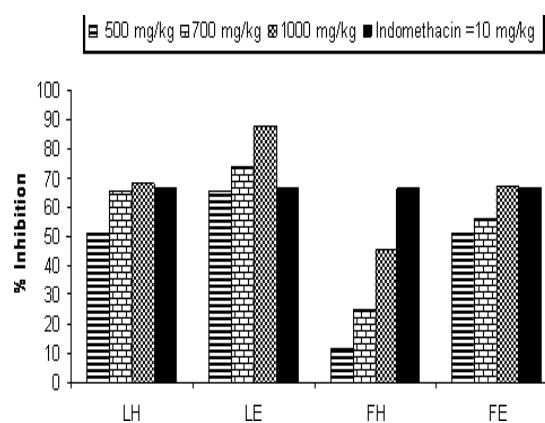
**Table 3.2.1:** Anti-inflammatory activity of various extracts of *Luffa cylindrica* in carageenan-induced rat paw inflammation.

Group	Dose (mg/kg) orally	Mean paw volume $\pm$ S. E. M. At 5 <sup>th</sup> in ml	Percent inhibition of oedema
Control	-----	0.97 $\pm$ 0.07	-----
IN	10	0.32 $\pm$ 0.01*	66.59
LE	500	0.33 $\pm$ 0.03*	65.13
	750	0.25 $\pm$ 0.03*	73.86
	1000	0.11 $\pm$ 0.02*	87.79
LH	500	0.47 $\pm$ 0.02	51.03
	750	0.33 $\pm$ 0.06*	65.63
	1000	0.30 $\pm$ 0.02*	68.28
FE	500	0.47 $\pm$ 0.02	50.96
	750	0.42 $\pm$ 0.01	56.26

	1000	0.32 ± 0.08*	67.0
FH	500	0.85 ± 0.02	11.71
	750	0.72 ± 0.02	24.9
	1000	0.52 ± 0.03	45.4

IN = Indomethacin; LE = Ethanolic extract of leaves; LH = Hexane extract of leaves; FE = Ethanolic extract of flowers; FH = Hexane extract of flowers. N = 6.\*P<0.05 is statistically significant values as compare to control using unpaired student's t-test.

**Figure 3.2.2:** Graphical presentation of antiinflammatory activity of *Luffa cylindrica*



#### 4. Discussion

From the results it is clear that all the extracts have antiemetic potential and are comparable with standard chlorpromazine. Although the results are significant but the mode of action is not known. However, as the oral copper sulphate induces emesis by peripheral action and peripheral 5- HT<sub>4</sub> plays an important role in copper sulfate induced emesis (Bhandari et al., 1991; Fukui et al., 1994), the extracts were able to effectively prevent its effect, it could be implied that these extracts have a peripheral antiemetic action.

The anti-inflammatory effects of plant extracts and natural products are frequently assessed by carrageenan-induced rat paw oedema (Panthong et al., 2003). Oedema development in carrageenan-induced paw oedema model in rats is represented by two phases (Vinegar et al., 1969). The first phase occurs within an hour of carrageenan injection and is partly due to the trauma of injection and also due to release of histamine and serotonin (Crunkhon and Meacock, 1971). Where as arachidonate metabolites (prostaglandins, leukotrienes) play a major role in the development of the second phase of reaction that is measured around 3-

hour time (Vinegar et al., 1969; Crunkhon and Meacock, 1971). The presence of prostaglandins in the inflammatory exudates from the injected foot can be demonstrated at 3-hour and period thereafter (Vinegar et al., 1969). Non-steroidal anti-inflammatory agents inhibit cyclooxygenase (COX-2) enzymes involved in prostaglandin synthesis (Robinson, 1997; Kulkarni et al., 2000). Based on these reports it is possible that the inhibitory effect of the hexane and ethanolic extracts of *Luffa cylindrica* (L.) Roem. on carrageenan-induced inflammation in rats could be due to inhibition of cyclooxygenase leading to inhibition of prostaglandin synthesis. Although the cyclooxygenase and lipoxygenase pathways are both involved in the inflammatory process, inhibitors of cyclooxygenase are more effective in inhibiting carrageenan-induced inflammation than lipoxygenase inhibitors (Flower et al., 1980). In our experiment, rats pre-treated with *Luffa cylindrica* (L.) Roem. showed a significant oedema inhibitory response at 5<sup>th</sup> hour following carrageenan injection. This result suggests that *Luffa cylindrica* extracts may act by suppressing the later phase of the inflammatory process by the inhibition of cyclooxygenase.

Phytochemical investigation of *Luffa cylindrica* reveals that leaves contain flavonoids (Schilling and Heiser, 1981) saponins (Liang et al., 1993 and 1996) and triterpenes (Nauking Institute of Materia Medica, 1980), while flowers contain flavonoids (Schilling and Heiser, 1981). Naturally occurring flavonoids and terpenes are reported as active principles against emesis (Kinoshita et al., 1996) where as flavonoids (Rotelli et al., 2003), saponins (Mahato et al., 1992) and triterpenes (Safayhi and Sailer, 1997), produce an inhibition of inflammation in the carrageenan-induced oedema. Therefore, it may be said that flavonoids, saponins and triterpenes may play some role in antiemetic and anti-inflammatory effects of these extracts. Further studies are required to determine the exact mode of action and the active compounds responsible for these effects.

#### Conclusion

In conclusion, extracts of *Luffa cylindrica* could have the potential to inhibit peripheral 5-HT<sub>4</sub> which plays an important role in copper sulphate induced emesis and inhibit cyclooxygenase, enzyme involved in the pathway of arachidonic metabolism responsible for inflammation. The extracts of *Luffa cylindrica* therefore appear to be a

promising source of useful antiemetic and anti-inflammatory agent.

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