Selected Pharmacological Studies of *Luffa operculata*

RONALD CHAMPNEY, NOEL M. FERGUSON*, and GARY G. FERGUSON[x]

Abstract □ An aqueous extract of *Luffa operculata* Cong. Cucurbitaceae (Cabacinha) was tested in mice for the following pharmacological effects: toxicity, analgesia ("grid shock" method), alteration of neuromuscular coordination (rotarod), and modification of barbiturate metabolism (hexobarbital sleeping time). The extract was evaluated in rats for analgesic effects (heat method); effects on electroshock seizure threshold; effects on blood pressure, pulse, electrocardiographic, and respiratory patterns; and anti-inflammatory activity. It was tested in guinea pigs for protective effect against histamine-induced bronchospasm. The extract was found to be inactive in all except analgesic studies in mice, in which a significant elevation of pain threshold was seen with 160 mg/kg ip. The 24-hr LD_{50} of the extract in mice was 160 mg/kg ip. A possible mechanism of the action is discussed.

Keyphrases □ *Luffa operculata*—pharmacological studies □ Pharmacological screening—extracts of *Luffa operculata* screened for activity

The saponins are found in some 500 plant species. Those of pharmacological importance are found in the families Rosaceae, Araliaceae, Liliaceae, Scrophulariaceae, and Polygalaceae (1).

Luffa operculata belongs to the family Cucurbitaceae (2). The active constituent was isolated and found to be isocucurbitacin B, which is believed to be responsible for its bitter taste (3). Other properties reported are foaming upon shaking, decongestant action on mucosa, hemolytic action on red blood cells, sternutatory effects (2), purgative, and abortifacient effects (4).

The extracts of *L. operculata* are highly toxic to mice and fish due to their hemolytic action on red blood cells (4). Because of this property, the saponin-containing plants are used as fish poisons in Africa and South America (1).

This study of *L. operculata* was begun because of interest in its decongestant property. An aqueous extract of the crude drug was studied, and a profile of pharmacological activity was obtained through the use of a screening program. Results of this study are reported here.

EXPERIMENTAL

Extraction—A quantity of 5.7 g of the ground, dried fruit of *L. operculata*[1] was boiled for 1.5 hr in 114 ml of distilled water. The remaining fluid was poured off, another 114 ml of distilled water was added to the marc, and the mixture was boiled for an additional 2.5 hr.

The resulting solution was filtered, and both filtrates were consolidated to form a dilution of 1:40. A volume of 28.5 ml of distilled water was added to the extract to make a final solution representing 1 g of crude drug in 25 ml (4%). This volume included water lost by evaporation.

Toxicity Studies—White, male, Swiss-Webster mice, weighing approximately 25 g, were injected intraperitoneally with the following arbitrarily chosen doses of 4% (40 mg/ml) *Luffa* extract: 0.05, 0.1, 0.2, 0.4, 0.8, and 1.6 ml. The mice were observed for effects such as sedation, excitation, salivation, defecation, and piloerection for 2 hr. Maximum drug effect appeared after 1 hr. A 1-hr period after injection was also allowed in most other studies, under the assumption that other responses would be at a high level after this period of drug incubation. The mice were again observed after 24 hr, and deaths were recorded. When an approximate LD_{50} dose was thus determined, 10 similar mice were injected with that dose and a more precise determination of the LD_{50} was made using similar techniques.

Rotarod Studies—Six white, male, Swiss-Webster mice, weighing approximately 25 g, were injected intraperitoneally (three controls and three experimentals). Each of three mice was treated intraperitoneally with 0.025, 0.050, and 0.075 ml, respectively, of a 4% (40 mg/ml) solution of *Luffa* extract; the three controls were injected intraperitoneally with equal volumes of distilled water.

All mice were placed on a rotarod having a speed of 6 rpm for 5 min, and the number of falls was recorded. The number of falls from the rotarod within a *short* time (to minimize the effect of fatigue) gives an estimate of the effect of the studied compound on neuromuscular coordination. Each mouse was given a 10-min rest between trials, and a total of four trials was performed.

Analgesia (Heat Method)—Twenty-eight white, male, Wistar rats were used (14 controls and 14 experimentals). The rats were weighed and injected intraperitoneally (14 with *Luffa* extract and 14 with distilled water). In each case, the dose was 80 mg/kg of the 4% extract.

The rats' tails were placed above the heating coil in a Dolorimeter[2] and the pain threshold was recorded (°C). The end-point was raising of the tail from the hot area ("tail flick" response).

Analgesia ("Grid Shock")[3]—Twenty white, male, Swiss-Webster mice were used in three separate trials. The mice were weighed and injected with 160 mg/kg ip of 4% *Luffa* extract or an equal volume of distilled water. Each mouse was placed in the grid shock apparatus for 1.5 hr. At 10-min intervals, the voltage was gradually increased from zero to the end-point (pain threshold), and the value thus obtained was recorded. The end-point was rapid and repeated lifting of a hindleg. Details of the testing procedure were previously reported (6).

Hexobarbital Sleeping Time—Twenty white, male, Swiss-Webster mice were used (10 experimentals and 10 controls) in each of three trials. A dose of 120 mg/kg ip of 4% *Luffa* extract or an equal volume of distilled water was used. After 1 hr of drug incubation, the mice were injected with 70 mg/kg ip of a 4-mg/ml solution of sodium hexobarbital. Sleeping time was considered to be the time interval from the point when the mouse lost the righting reflex to the point when the righting reflex was regained. This procedure was repeated two additional times.

Electroshock Seizure Threshold—Twenty white, male, Wistar rats were used. Each was shocked with corneal electrodes to determine the threshold for maximal tonic seizure. Normal saline solution was placed on each cornea to assure proper conductance. Thresholds were measured (milliamperes) with an electroshock apparatus[2], which provided a shock of 110 v with variable amperage and a duration of 0.2 sec.

After maximal tonic thresholds were determined, 10 rats were injected with 2 ml/kg ip of distilled water and 10 were injected with 80 mg/kg ip of 4% *Luffa* extract. After 1 hr, the rats were then shocked at their predetermined thresholds to observe any change.

Blood Pressure, Electrocardiographic (ECG), Pulse, and Respiration Studies—Ten white, male, Wistar rats were used.

[1] Collected in Northeast Brazil and identified by Dr. Lauro Xavier. A voucher specimen is deposited at the Universidade Federal Da Paraiba, Joao Pessoa, Paraiba, Brazil.

[2] Metro Industries, Farmingdale, N.Y.

[3] Consisting of a wooden box having a floor composed of brass rods wired in parallel to deliver a mild electric shock to the animal's feet.

Normal tracings for indirect blood pressure, ECG, pulse, and respiration were determined with the Physiograph[4], using the pneumatic pulse transducer[4], high gain preamplifier[4], and impedance pneumograph[4] in the measurement of the respective parameters. Respiration, pulse rate, and ECG were monitored using needle electrodes placed beneath the skin of conscious rats, which were restrained in Physiograph small animal housings[4]. Indirect blood pressure was obtained from the tail with the pulse transducer using an electrosphygmomanometer[4] which automatically inflated and deflated a pressure cuff around the tail and allowed for measurement of systolic blood pressure. Seven rats were then injected with 40 mg/kg iv of 4% *Luffa* extract, and three rats were injected with 160 mg/kg iv of 4% *Luffa* extract. Each animal was injected three times with its particular dose of extract. Tracings of blood pressure, ECG, pulse, and respiration were again obtained following each injection to observe any alterations in patterns.

Foot Volume Plethysmography—Fifteen white, male Wistar rats were used in each of two trials. In the first trial, following initial measurement of foot volumes, 15 rats were injected with 0.1 ml of 1% silver nitrate solution through the Achilles tendon of the left hindfoot to induce swelling of the foot pad. After 18 hr, the foot volume was again measured (in arbitrary units) with the plethysmograph[5] to confirm the presence of pedal edema. Five rats were injected with a solution of oxyphenbutazone (50 mg/kg ip solubilized in 5% polyethylene glycol), which served as a reference anti-inflammatory compound. Five rats were injected intraperitoneally with 5% polyethylene glycol solution in a volume equal to that of reference compound. Five rats were injected with 80 mg/kg ip of 4% *Luffa* extract. After a 1-hr incubation period, each rat's foot volume was measured at 1-hr intervals for 2 hr.

The second trial was conducted in the same manner except for the following changes: 0.2 ml of 1% silver nitrate solution, 1 ml/kg of distilled water as control, 1 mg/kg of dexamethasone to replace oxyphenbutazone, and 160 mg/kg of 4% *Luffa* extract.

Foot volume was measured hourly for 4 hr after the 1-hr incubation period. The procedure is similar to that previously reported (5).

Histamine Antagonism—Eight adult, male guinea pigs were weighed and then divided into two groups of four (controls and experimentals). The experimental animals received 120 mg/kg ip of 4% *Luffa* extract 30 min prior to testing, and controls received 3 ml/kg ip distilled water in an identical treatment. After incubation, each animal was placed in an airtight chamber into which was sprayed 0.5 ml of 0.5% histamine diphosphate solution. The animals were observed for signs of bronchoconstriction, hypoxia, or asphyxia.

RESULTS AND DISCUSSION

Toxicity Studies—The following observations were made during the test period. Regardless of the dose of *Luffa*, the effects were similar. At first, defecation increased followed in approximately 30 min by watery diarrhea. Spontaneous motor activity appeared to decrease. All *Luffa*-treated mice remained in a crouched position with eyes closed and head lowered. Piloerection was present in some animals. Some mice lost the righting reflex. Controls appeared normal at the times these observations were made. The 24-hr LD_{50} was approximately 160 mg/kg ip of 4% *Luffa* extract in mice.

Rotarod Performance; Analgesia (Heat Method); Hexobarbital Sleeping Times; Electroshock Seizure Threshold; Blood Pressure, ECG, Pulse, and Respiration Studies; Foot Volume Plethysmography; and Antihistamine Studies—No significant results were observed ($p > 0.05$).

Analgesia (Grid Shock)—The grid shock testing showed an elevated pain threshold for up to 1.5 hr after injection of *Luffa* extract. Statistical analysis showed a significant difference as compared to controls (Table I).

Table I—Pain Thresholds in Control and Treated Animals[a]

Time after Injection, min	Control	Experimental	T^b
10	13	17	3.7
30	14	20	2.7
50	16	24	2.0
70	15	26	3.2
90	17	28	3.7

[a] Each value, expressed in volts, represents the average of nine individual determinations. For details of treatment, see text. [b] $p < 0.05$.

The absence of activity of the extract in the cardiovascular, anti-inflammatory, and antihistaminic studies was surprising, considering the previously reported stimulant action of the drug on toad heart, guinea pig ileum, and rabbit duodenum; its pressor effects in cats; and its colloquial use in the treatment of nasal congestion (2, 4). Most drugs that are beneficial in the treatment of nasal congestion act by means of adrenergic stimulation, producing vasoconstriction of the capillaries in the nasal mucosa, or by antihistaminic or anti-inflammatory effects, resulting in reduced engorgement of the nasal mucosa (7). While the degree of engorgement of the nasal mucosa and resultant resistance to nasal air passage is difficult to evaluate in animals, the studies performed should have provided a parallel means of evaluating the effect of *Luffa* extract on these parameters in terms of effects on certain cardiovascular functions, changes in response to histamine, or modification of experimentally induced inflammatory edema. Thus, if the drug has marked vasoconstricting effects, some elevation in blood pressure or change in pulse rate would be expected in animal studies. Likewise, if the drug acts as an anti-inflammatory agent, modification of silver nitrate-induced pedal edema in rats would be expected. If the primary action of the drug is as an antihistaminic, partial or complete protection of guinea pigs from histamine-induced bronchospasm should be seen. If the action or a portion of the action of *L. operculata* is as an analgesic, it must be assumed to be of a mild degree since near lethal doses were required for the effect in mice and this effect was not seen in the heat method studies but required the more sensitive grid shock method for its detection.

REFERENCES

(1) N. M. Ferguson, "A Textbook of Pharmacognosy," Macmillan, New York, N.Y., 1956, pp. 87, 88.

(2) J. B. da Silva, *Rev. Fac. Farm. Bioquim., Univ. Sao Paulo*, **2** (2), 15(1964); through *Chem. Abstr.*, **63**, 12969e(1965).

(3) F. J. A. Matos and O. R. Gottlieb, *An. Acad. Brasil. Cienc.*, **39** (2), 245(1967).

(4) G. S. G. Barros, F. J. A. Matos, J. E. V. Vieira, M. P. Sousa, and M. C. Medeiros, *J. Pharm. Pharmacol.*, **22**, 116(1970).

(5) S. Margolin, *Excerpta Med. Found.*, **82**, 214(1965).

(6) R. E. Stull, N. M. Ferguson, and G. G. Ferguson, *J. Pharm. Sci.*, **60**, 1221(1971).

(7) "The Merck Manual," Merck and Co., Rahway, N.J., 1962, p. 9.

ACKNOWLEDGMENTS AND ADDRESSES

Received September 28, 1973, from the *School of Pharmacy, Northeast Louisiana University, Monroe, LA 71201*

Accepted for publication February 5, 1974.

* Present address: College of Pharmacy, University of Houston, Houston, TX 77004

x To whom inquiries should be directed.

[4] Narco Industries, Houston, Tex.

[5] Plethysmographe V3, Ugo Basile, Milan, Italy. Volume is measured by mercury displacement, using a balanced bridge electronic system and meter-type dial.