Toxicological and Pharmacological Properties of Sea Pansy, *Renilla mulleri*

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Abstract □ *Renilla mulleri* specimens, collected from the Mississippi Gulf Coast, were homogenized and centrifuged, and the supernatant liquid was lyophilized. Mice injected with 100–250 mg./kg. i.p. of the lyophilized extract dissolved in phosphate buffer (pH 6.5) became lethargic, and most of them died within 24 hr. The LD_{50} in mice was 160 mg./kg. A dose of 10–20 mg./kg. i.v. produced a fall in blood pressure and a distinct bradycardia in anesthetized cats. These effects were not abolished by either bilateral vagotomy, atropinization, or adrenergic blockade. At higher doses, the extract produced irregularities of heart actions. In isolated rabbit heart preparations, the extract produced a decrease in the rate and amplitude of cardiac contractions and the coronary outflow. The extract had a relaxant effect on isolated rabbit duodenum, guinea pig ileum, and rat uterus. The effect of the extract on the blood pressure, heart, and isolated smooth muscles is due to a direct myotropic effect.

Keyphrases □ *Renilla mulleri*—toxicology, pharmacology □ Sea pansy—toxicology, pharmacology □ Toxicology—*Renilla mulleri* (sea pansy)

The class *Anthozoa* is comprised of two subclasses (1): the *Alcyonaria*, which includes the soft corals, sea fans, sea pens, and sea pansies; and *Zoantharia*, which includes the sea anemones and true corals.

A sea pansy colony consists of a flattened, kidney-shaped rachis, on the upper surface of which the polyps are born (2). The short-stemmed sea pansy, *Renilla mulleri*, is common in the northern Gulf of Mexico and extends southward to Brazil (3). There are no known published data on the biotoxicity of *Alcyonarians* (4). Therefore, this study was undertaken to investigate the toxicity of the sea pansy (*R. mulleri*) obtained from the Gulf of Mexico.

MATERIALS AND METHOD

R. mulleri[1] specimens were found to be abundant around Cat Island, Ocean Springs, Miss. They were taken at a salinity and temperature range of 24.9–30.0 p.p.t. and 15.0–24.9°, respectively. Twenty specimens, with total weight of 120 g., were homogenized in a chilled homogenizer for 10 min. The homogenate was centrifuged for 10 min. at 9000 r.p.m., and the supernatant liquid (light red in color) was lyophilized. The lyophilized material (dark red) was dissolved either in distilled water or 0.1 *M* phosphate buffer (pH 6.5) for pharmacological studies.

Toxicity Studies—General acute toxicity and various toxic manifestations were studied on male albino mice. Mice weighing 15–20 g. were randomly grouped with five animals in each group. They were administered intraperitoneally with the aqueous solution of the lyophilized material and housed in individual cages provided with food and water. The toxic symptoms and behavioral changes exhibited by the animals were observed for 48 hr. The LD_{50} was estimated according to the method of Horn (5).

Pharmacological Studies—*Locomotor Activity*—Albino mice[2] were used for motor activity studies. An automatic timer provided a 12-hr. dark, 12-hr. light schedule in the room housing the animals; air conditioning maintained room temperature between 22 and 23° (72 and 76°F). All mice were allowed to adapt to these environmental conditions for a minimum of 7 days before being used in the experiment.

Measurement of spontaneous locomotor activity was recorded by means of three photocell activity cages (actometers)[3]. Each actometer was covered with a sound-attenuating box. Electromechanical counters registered an activity count each time the animal traversed one-sixth the circumference of the runway. By means of a stopwatch and the counter, counts were taken every 15 min. for 2 hr. The statistical evaluation of the data in this study involved use of one-factor analysis of variance (6).

Blood Pressure and Respiration—The effects of the toxin on blood pressure, heart, and respiration were studied in anesthesized cats. Overnight fasted cats (2.5–3.5 kg.) of both sexes were anesthetized with 35–40 mg./kg. sodium pentobarbital intraperitoneally. The right common carotid artery was exposed at the neck region and cannulated with a polyethylene cannula, which was connected to a previously calibrated (linear core) pressure transducer[4] for the blood pressure recording. Respiration was recorded from chest electrodes, which were connected to an impedance pneumograph[4]. While recording respiration, the same electrodes were connected to an ECG preamplifier for obtaining standard Lead II electrocardiograms. The ECG preamplifier was calibrated in such a manner that each deflection represented 1 mv. in amplitude and 10 mm. in height. The pressure transducer, pneumograph, and ECG preamplifier were connected to a physiograph[5] by standard transducer cables for recording. The ECG patterns were interpreted according to the description of Burch and Winsor (7) and Friedman (8).

All injections were made through the right femoral vein, which was exposed and cannulated with a venous polyethylene cannula. The cholinergic activity of the toxin was studied in bilaterally vagotomized and completely atropinized animals; *e.g.*, both vagi were cut at the cervical level and the animals were injected with atropine (3 mg./kg. i.v.). Complete parasympathetic blockade was ensured when no response was obtained with a test dose of acetylcholine (5 mcg./kg. i.v.).

Autonomic ganglia were blocked by the administration of hexamethonium bromide (5 mg./kg. i.v.) in three successive doses. The animals were injected with phenoxybenzamine (3 mg./kg.) for complete blockade of peripheral sympathetic α-receptors, which was ensured by observing reversal of the action of epinephrine (5 mcg./kg. i.v.) on the blood pressure. β-Receptors were blocked by dichloroisoproterenol (4 mg./kg. i.v.). For determining the action on the baroreceptors of the carotid sinus, carotid occlusion was performed three times for 30 sec., before and after the administration of the toxin.

Isolated Heart Preparation—The effect of the toxin on isolated rabbit heart was studied to investigate the effects on the cardiac muscle, using Langendroff's technique as modified by Anderson and Craver (9). The chest was opened and the heart removed. It was placed into an oxygenated Locke solution and gently squeezed to remove blood from the aorta. The aorta was freed from its attachment to the pulmonary artery, and a cannula was tied to it. After the cannula was fixed in place, a hook with thread was fixed on the tip of the ventricle and the heart perfused. The thread was passed through a pulley and attached to a myograph[6], which was connected to the physiograph by standard transducer cables for recording. A constant pressure was maintained throughout the perfusion.

[1] Identified by Dr. Gordon Gunter, Gulf Coast Research Laboratory, Ocean Springs, Miss.
[2] Southern Animal Farm, Prattville, Ala.
[3] Model R064, Woodard Research Corp., Herndon, Va.
[4] E & M Instrument Co., Inc., Houston, Tex.
[5] Model DMP-4A, NARCO.
[6] Model B-655, E & M Instrument Co., Inc., Houston, Tex.

Table I—Effects of *R. mulleri* Toxin on Locomotor Activity

Minutes	Activity Counts/15 min.[a] I (20 mg./kg.) Control	I (20 mg./kg.) *Renilla*	II (40 mg./kg.) Control	II (40 mg./kg.) *Renilla*
15	453.6 ± 82.6	178.5 ± 35.7[b]	592.1 ± 83.8	206.6 ± 42.7[c]
30	317.1 ± 75.4	83.1 ± 36.6[b]	343.0 ± 41.0	135.0 ± 42.9[c]
45	269.8 ± 45.6	81.8 ± 33.6[b]	256.6 ± 24.4	62.0 ± 20.6[c]
60	321.6 ± 70.8	72.8 ± 17.7[b]	202.3 ± 17.7	78.3 ± 25.7[c]
75	201.3 ± 38.6	71.3 ± 15.6[b]	166.8 ± 18.1	65.6 ± 18.2[c]
90	221.5 ± 31.0	55.3 ± 11.0[c]	169.6 ± 21.5	50.0 ± 23.4[c]
105	213.5 ± 60.7	50.3 ± 12.3[b]	158.6 ± 25.8	26.1 ± 17.4[c]
120	203.0 ± 32.9	31.1 ± 7.9[c]	197.3 ± 14.0	16.0 ± 3.3[c,d]
Total	2266.5 ± 30.0	622.5 ± 119.7[c]	2080.0 ± 153.8	639.8 ± 146.3[c]

[a] Each value represents the mean ± the standard error for a group of six mice. [b] $p < 0.01$ when compared with the control group. [c] $p < 0.05$ when compared with the control group. [d] $p < 0.05$ when 20-mg./kg. dose was compared with 40-mg./kg. dose.

Isolated Hind Quarter of the Rat—The action of the toxin on the blood vessels was recorded on the hind-quarter preparation of albino rats (10). The animals were sacrificed and eviscerated. A cannula was tied in the abdominal aorta, and the body wall and vertebral column were cut above the point of cannulation.

The hind quarter of the rat was then laid on a piece of muslin attached to a wire, which rested on a glass funnel. A marriotte bottle full of Ringer's solution was connected by a rubber tubing to the cannula. The vessels of the rat were perfused until the perfusate was free from blood. The outflow from the vessels passed through the muslin and the funnel dropwise and was measured by counting the drops per minute. All injections were made directly into the connecting rubber tubing.

Isolated Smooth Muscle—Rabbits weighing 1–2 kg. and guinea pigs weighing 300–500 g. of both sexes were used. The animals were sacrificed, and the intestines were exposed. An actively contracting loop of ileum (guinea pig) or duodenum (rabbit), 4–6 cm. in length, was selected and cut. This section was suspended in a 25-ml. bath containing Tyrode's (guinea pig ileum) or Ringer's solution (rabbit duodenum). One end of the loop was tied to the lower hook of the holder and the other end was attached to the myograph. The preparation was suspended in an organ bath of Tyrode's solution, which was maintained at 37° and oxygenated with 95% O_2 and 5% CO_2.

RESULTS

Toxicity Studies—Mice injected with various doses of the toxin became lethargic within a few minutes. The animals remained motionless and later developed respiratory distress; some died within 30 min. The LD_{50} was estimated to be 160 mg./kg.

Pharmacological Studies—*Locomotor Activity*—As shown in Table I, *Renilla* toxin reduced the motor activity of mice. The absorption of the toxin seemed to be rapid, as was indicated by the significant reduction in motor activity in the first 15 min. Both 20 and 40 mg./kg. significantly reduced the motor activity; however, there was no significant difference between the two doses except at the 120-min. interval.

Effect on Blood Pressure and Respiration of the Cat—Five experiments were carried out on anesthetized cats with different doses of toxin, ranging from 20 to 40 mg./kg. A 20–60 mm. Hg decrease in the arterial blood pressure was observed (Fig. 1A). There was a marked initial fall in the blood pressure, which then stabilized at a level of 10–20 mm. Hg below the baseline. A single dose produced a persistent fall in blood pressure which lasted for 7 hr. The decrease in the blood pressure showed a consistent dose–response relationship. Slowing of the pulse rate was a consistent observation, occurring within 5 sec. after the injection of the toxin (Fig. 1A). All of the doses caused a marked change in respiratory rate, which remained above the normal level throughout the experiment (Fig. 1B).

The fall in blood pressure was not modified by bilateral vagotomy, atropinization, ganglionic blockade, sympathetic blockade, or treatment with antihistaminic agents. A similar blood pressure response to carotid occlusion was obtained before and after the administration of the toxin.

Effect on Electrocardiogram of the Cat—With a 20-mg./kg. dose, there was an increase in the P-R interval, R wave amplitude, and S-T segment, and there was an inversion of the T wave (Fig. 1D). When a 30-mg./kg. dose was used, there was a further increase in the R wave amplitude and P-R interval, depression in the S-T segment, and deep inversion of the T wave (Fig. 1E). This was followed by a 1:3 complete heart block with 40 mg./kg. (Fig. 1F).

Effect on Isolated Rabbit Heart—The control values (five observations) for coronary flow rate (2.8 ± 0.4 ml./min.), heart rate (101 ± 7 beats/min.,), and amplitude of cardiac contractions (21.0 ± 0.5 mm. deflection) were consistent throughout the experiment. The injection of the toxin was made into the coronary perfusion

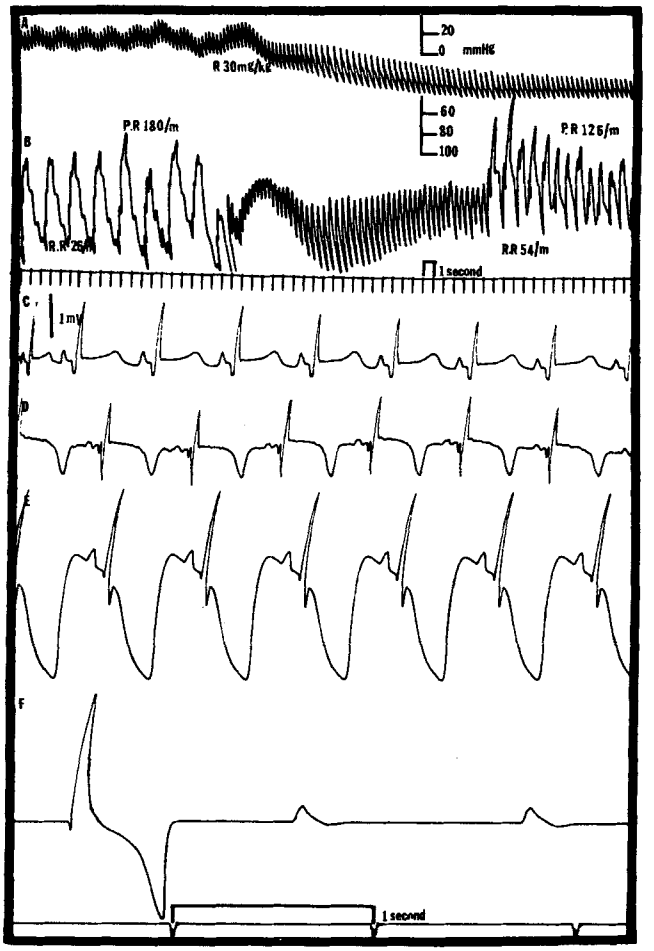

Figure 1—*Effect of* R. mulleri *toxin on: A, blood pressure*; B, respiration; C, ECG control; D, E, F, various changes in ECG at the dose levels of 20, 30, and 40 mg./kg. of the toxin. Key: R =* R. mulleri *toxin, PR = pulse rate, RR = respiration rate, and mv. = millivolt. *No initial blood pressure is shown on the scale; instead the scale is divided in such a manner that initial blood pressure reads as 0.00 mm. Hg. The pressure transducer was calibrated for a 100-mm. Hg rise or fall in the blood pressure, which is shown on the scale.*

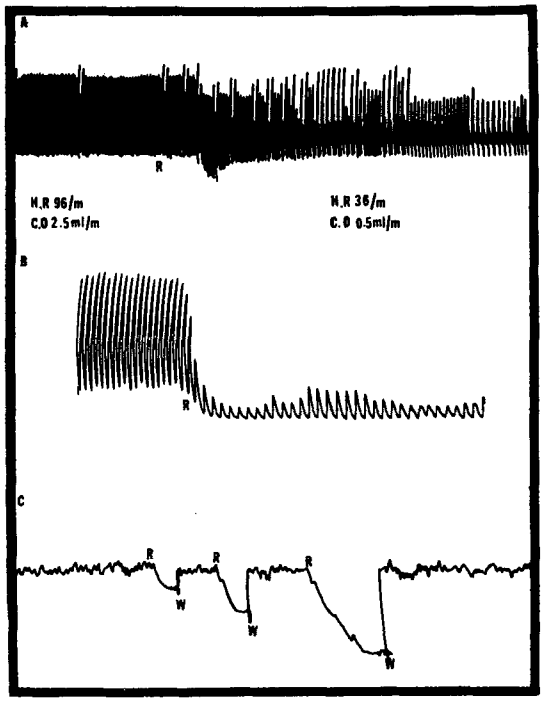

Figure 2—*Effects of sea pansy* (R. mulleri) *extract on the isolated rabbit heart, isolated rabbit duodenum, and isolated guinea pig ileum. Key: A = effect of 2-mg. dose of the toxin injected into the perfusion fluid of the isolated heart preparation; B = effect of 200 mcg./ml. of the toxin on the isolated rabbit duodenum; C = effect of 100, 200, and 300 mcg./ml. of the toxin on the isolated guinea pig ileum; R* = R. mulleri *extract; HR = heart rate; CO = coronary outflow; /m = per minute; and W = wash.*

circuit. With a dose of 1 mg., there was an immediate decrease in the coronary outflow and heart rate to 2.1 ± 0.2 ml./min. and 72 ± 6 beats/min., respectively; however, there was no change in the amplitude. The effects persisted over 10 min. and gradually returned to normal.

A 2-mg. dose had a more persistent effect, lasting for more than 30 min. (Fig. 2A). The changes in the coronary outflow and heart rate from control were 2.7 ± 0.3 ml./min. and 38 ± 4 beats/min., respectively. The amplitude fluctuated without any regular pattern, probably because of some irregular beats of the heart at this dose level. When a 3-mg. dose was used, there were drastic changes in all three parameters, resulting in irregular ventricular contractions and decreased amplitude; ultimately the heart stopped within 10 min.

Effect on Isolated Hind Quarter of the Rat—Two doses of the toxin, 2 and 4 mg., injected directly into the perfusate caused a significant vasodilation. The increase in the number of drops from control (12 ± 2 drops/min.) was 19 ± 3 and 27 ± 2 drops/min., respectively. The effect of the 4-mg. dose lasted for more than 30 min.

Effect on Isolated Rabbit Duodenum—The toxin in the 200-mcg./ml. dose reduced the pendular movements and relaxed the rabbit duodenum. The effect was so persistent (Fig. 2B) that repeated washings could not bring the intestine strip back to the baseline.

Effect on Isolated Guinea Pig Ileum—The toxin at doses of 100, 200, and 300 mcg./ml. relaxed the guinea pig ileum (Fig. 2C). Doses higher than 300 mcg./ml. relaxed the muscle to such an extent that repeated washings could not bring it back to normal.

DISCUSSION

The *Renilla* toxin was found to be toxic to mice, having an LD_{50} of 160 mg./kg. In gross behavioral observation and motor activity studies, the toxin appeared to have CNS depressant action.

The toxin at various dose levels produced a persistent fall in blood pressure. This effect was not blocked by cholinergic, adrenergic, and ganglionic blockers, nor could antihistaminic agents abolish the effect. The speculation that the fall in the blood pressure produced by the toxin was due partially to a direct effect on the peripheral blood vessels was confirmed by the vasodilatory effect observed in the studies on isolated hind quarter of the rat.

ECG recordings from cats injected with the toxin showed prolongation of the P-R interval, which was due to a slow transmission of impulses from the A-V node. A prolonged Q-R-S complex suggested a depression of the rate of ventricular depolarization, and a depressed T wave indicated cardiac ischemia. Higher doses depressed the Q-R-S complex and further inverted the T wave. This was followed by 1:2 and 1:3 complete atrioventricular block, which was due to extremely poor conduction of impulses from the atrium into the ventricle.

On the isolated heart preparation, the toxin in smaller doses reduced the heart rate, amplitude of contraction, and coronary outflow. At higher doses, the toxin caused irregularities of cardiac contractions. The fall in the blood pressure seems to be attributable to two factors: (*a*) decreased cardiac output caused by the effect of the toxin on the heart, depressing contractility, excitability, and automaticity; and (*b*) vasodilation, as indicated by an increase in outflow in the rat's hind-leg preparation.

The toxin damaged the heart at higher doses, as indicated in both *in vivo* and *in vitro* experiments. It appeared that the toxin acted both on the conduction system and cardiac muscle, as indicated by cardiac ischemia and complete heart block.

The toxin relaxed rabbit duodenum and guinea pig ileum, but no effect on the rat uterus or frog skeletal muscle was indicated.

SUMMARY

R. mulleri (sea pansy) was toxic to mice, having an LD_{50} of 160 mg./kg. The toxin reduced the motor activity in mice. It produced a persistent fall in the blood pressure, had a vasodilatory effect on the isolated hind quarter of the rat, and caused relaxation of extravascular smooth muscles. The toxin caused cardiac ischemia and 1:2 and 1:3 complete atrioventricular block in intact animals, as indicated by ECG recordings. The fall in blood pressure could be attributed to two factors: (*a*) decreased cardiac output caused by the effect of the toxin on the heart, depressing contractibility, excitability, and automaticity; and (*b*) vasodilation, as indicated by increased outflow in the rat hind-quarter perfusion test.

REFERENCES

(1) L. H. Hyman, "The Invertebrates: Protozoa through Ctenophora," vol. 1, McGraw-Hill, New York, N. Y., 1940, p. 103,

(2) W. Crowder, "Between the Tides," Vail Ballou Press. Binghamton, N. Y., 1931.

(3) P. S. Galstoff, "Gulf of Mexico, Its Origin, Waters and Marine Life," U. S. Government Printing Office, Washington, D. C., 1954, p. 251.

(4) B. W. Halstead, "Poisonous and Venomous Marine Animals of the World," vol. 1, U. S. Government Printing Office, Washington, D. C., 1965, p. 314.

(5) H. J. Horn, *Biometrics*, **12,** 311(1956).

(6) L. A. Edwards, "Experimental Design in Psychological Research," Holt, Rinehart and Winston, New York, N. Y., 1970.

(7) G. E. Burch and T. Winsor, "A Primer of Electrocardiography," Lea & Febiger, Philadelphia, Pa., 1954.

(8) H. H. Friedman, "Outline of Electrocardiography," McGraw-Hill, New York, N. Y., 1963, p. 99.

(9) F. F. Anderson, and B. N. Craver, *J. Pharmacol. Exp. Ther.*, **93,** 135(1948).

(10) J. H. Burn, "Practical Pharmacology," Blackwell Scientific Publications, Oxford, England, 1952, p. 65.

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