Preliminary Study of Potential Antiarrhythmic Effects of *Crataegus monogyna*

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Abstract □ Crude extracts of the bark and leaves of *Crataegus monogyna* were tested in rabbits for potential antiarrhythmic activity. Four fractions (A contained the petroleum ether solubles, B contained the 95% ethanol solubles, C contained the chloroform solubles, and D contained the water solubles), which were infused 5 min after arrhythmia had been induced by intravenous injection of 10 μg/kg of aconitine, did not cause the arrhythmia to revert to normal cardiac rhythm. However, when the maximal tolerated amounts of either Fraction B or D of the bark or leaves were first infused, higher doses of aconitine than those used in control animals were required to generate arrhythmias in the treated rabbits. The present study showed that the extracts from bark and leaves of *C. monogyna* possess a prophylactic antiarrhythmic activity.

Keyphrases □ *Crataegus monogyna* L. (Rosaceae)—antiarrhythmic effects of crude extracts of bark and leaves □ Medicinal plants—*C. monogyna*, crude extracts of leaves and bark screened for antiarrhythmic effects □ Antiarrhythmic activity—*C. monogyna* leaf and bark extracts screened

Several species of the genus *Crataegus*, commonly called Hawthorn, have been studied for their potential medicinal value. Claims of pharmacological activity range from curative agents in hypertension to the treatment of certain cardiac disorders. As early as 1896, Jennings (1) advocated the use of tincture of *Crataegus oxyacantha* as an adjuvant to, and even a substitute for, *Digitalis* in the treatment of cardiac disorders. A different species, *Crataegus pentagyna*, was reported by previous investigators to possess an effect on the isolated mammalian heart (2–4). A later report (5) referred to the antiarrhythmic effectiveness of *C. pentagyna* against aconitine-induced arrhythmias in rabbits.

In 1949, Neugebauer (6) reported that *Crataegus monogyna* contained a substance that was intensely active on the heart. Some years later, Andreeva (7) showed that extracts of *C. monogyna* elicited sedative activity and a positive inotropic effect on the frog heart, the major part of the activity being found in infusions of the flowers and bark. Nikolov and Ivanov (8), in 1969, reported that a flavonoid mixture from *C. monogyna* improved cardiac activity in experimental animals.

Although other *Crataegus* species have been shown to prevent cardiac arrhythmias (2–4), this effect has not been demonstrated for *C. monogyna*. Thus, it was decided to carry out a preliminary pharmacological investigation on extracts of the leaves and bark of *C. monogyna* to determine whether they possess antiarrhythmic activity.

EXPERIMENTAL[1]

Preparation of Extracts—One kilogram of coarsely powdered leaves of *C. monogyna* was continuously extracted with petroleum ether (bp 30–60°) in a soxhlet extractor until the final extract was colorless. Evaporation of the petroleum ether *in vacuo* yielded 2 g of an oily residue.

The defatted powder was air dried and macerated for 24 hr with 95% ethanol and percolated slowly until the eluate was colorless. Evaporation of the ethanol *in vacuo* yielded a 35-g residue, of which 12 g was set aside for testing. The remaining 23 g was taken up in 100 ml of water and extracted four times with 50-ml portions of chloroform. After the separation of the two layers, the chloroform extract was dried over anhydrous sodium sulfate and taken to dryness *in vacuo*. The yield was 12.3 g. Lyophilization of the aqueous fraction yielded 9.6 g of residue. Fraction A contained the petroleum ether solubles, Fraction B contained the 95% ethanol solubles, Fraction C contained the chloroform solubles, and Fraction D contained the water solubles.

Similarly, 1 kg of coarsely powdered bark of *C. monogyna* yielded 0.5 g of Fraction A and 40 g of Fraction B, of which 25 g was partitioned between chloroform and water. Fraction C weighed 14 g while Fraction D weighed 11 g.

Solubilization of Plant Extracts—Solutions of the various fractions of *C. monogyna* used for infusion in the experimental animals were prepared by dissolving the extract in an appropriate solvent to obtain a concentration of 50 mg/ml, from which dilutions were made as needed. One gram each of Fractions D and B of the bark and leaves, respectively, was dissolved in 20 ml of a solvent mixture. The mixture contained a sufficient amount of dimethyl sulfoxide to dissolve the solid material, and the final volume was made up to 20 ml with distilled water to obtain the desired concentration (50 mg/ml).

Preparation of Animals—Adult female albino rabbits, weighing 2.0–3.0 kg, were used. Each animal was anesthetized with 25 mg/kg iv sodium pentobarbital[2]. A femoral artery and vein were each cannulated for monitoring arterial blood pressure and for drug administration, respectively. Conventional limb lead II was used to monitor the ECG, from which heart rate was derived. Antiarrhythmic activity of *C. monogyna* was tested against aconitine[3]-induced arrhythmias, using a modification of the method introduced by Scherf (9). Initially, the arrhythmogenic dose of aconitine was established by intravenous sequential injections of 10 μg/kg at intervals of 15 min until arrhythmia developed. When the induced arrhythmia was well sustained, about 30 ml or more of the test solution of the crude extract of *C. monogyna* was infused intravenously at a rate of 0.75 ml/min by means of an infusion pump[4]. Infusion was discontinued when normal sinus rhythm was restored or when death resulted.

In another group of rabbits, 30 ml of the appropriate crude frac-

[1] The leaves and bark of *C. monogyna* L. (Rosaceae) were collected from plants cultivated at the Morton Arboretum, Lisle, Ill., in July 1973. The plant material was air dried and milled to a coarse powder. The identity of this plant material was confirmed by Mr. Floyd Swink of the Morton Arboretum, and a voucher specimen (SP-2510) from the collection has been deposited in the herbarium of the Department of Pharmacognosy and Pharmacology, University of Illinois at the Medical Center.

[2] Sodium Nembutal, Abbott.

[3] Pfaltz Bauer Inc., Flushing, N.Y.

[4] Harvard compact infusion pump model 975.

Table I—Aconitine-Induced Arrhythmia after *C. monogyna* Infusion

Extract Infused (Concentration)	Number of Animals	Amount of Extract Infused	Total Amount of Aconitine Injected	Number of Animals Exhibiting Arrhythmias/Number Tested	Protection, %
Control	5		10 ± 2.0[b]	5/5	0
Fraction D of bark (10 mg/ml)	8	375 ± 50[a]	53 ± 16[b]	3/8	63
Fraction B of bark (10–20 mg/ml)	6	340 ± 88[a]	60 ± 12[b]	2/6	67
Fraction D of leaves (10–20 mg/ml)	6	300 ± 1.0[a]	35 ± 8[b]	3/6	50
Fraction B of leaves (10–20 mg/ml)	2	600 ± 1.0[a]	35 ± 10[b]	2/2	0

[a] Mean ± standard error (milligrams). [b] Mean ± standard error, micrograms per kilogram aconitine.

tion (10–20 mg/ml) was infused into the experimental animals at a rate of 0.75 ml/min, while the controls received either equivalent amounts of saline or the appropriate solvent in which the fraction was dissolved (a mixture of dimethyl sulfoxide and water). Thirty minutes after the infusion was completed, each animal was challenged with the test dose of aconitine (10 μg/kg) at regular intervals of 15 min for no longer than 90–95 min or until arrhythmia developed. The index of protection was taken as a lack of the development of abnormal cardiac rhythm during aconitine injection. The total amount of aconitine administered was then recorded.

RESULTS AND DISCUSSION

The arrhythmogenic dose of aconitine determined in five rabbits was 10.0 ± 2.0 μg/kg. These arrhythmias occurred within 9–12 min after the initial injection of aconitine. The cardiac disturbances included isolated ventricular extrasystoles at the start, which developed into runs of ventricular tachycardia and, in a few instances, ventricular fibrillation. Only in one animal did a reversion of the arrhythmia to normal sinus rhythm occur with the infusion of Fraction D of the bark of *C. monogyna* (50 mg/ml). Fraction D of the leaves and Fractions B of the leaves and bark failed to abort the aconitine-induced disturbance. None of the Fractions A, B, and C of *C. monogyna* bark and leaves tested reversed aconitine-induced arrhythmia.

In the prophylactic test for antiarrhythmic activity, certain fractions of *C. monogyna* demonstrated protection, while others did not (Table I). Death occurred almost instantaneously upon start of the infusion of either Fraction A or C from the bark or leaves. These fractions did not afford any degree of protection at any dose. Considerable protection was observed with Fraction D of the bark since minimal arrhythmias developed with the injection of aconitine. Moderate protection was afforded by Fraction D of the leaves and Fraction B of the bark; the arrhythmia seen was of short duration and occurred only after very high doses of aconitine had been injected. Since earlier experiments had established that the mean arrhythmogenic dose of aconitine in five rabbits was 10.0 ± 2.0 μg/kg iv, the large amounts of aconitine which the rabbits tolerated after the infusion of these fractions suggested a protective antiarrhythmic effect of the principles in both the leaves and bark (Table I).

Only two of 22 rabbits infused with the various fractions (especially Fraction D) were completely protected since there was no occurrence of arrhythmia after infusion of *C. monogyna*. However, the other animals exhibited a moderate degree of protection since large amounts of aconitine (greater than control amounts) did induce mild arrhythmia in a few of these treated rabbits.

The weak protective activity observed in *C. monogyna* may be attributed partly to the fact that all studies in this laboratory were conducted with crude extracts of the various fractions. Thus, the presence of extraneous substances may have masked the true activity of the principles present in the plant parts tested. However, the test conducted (5) with isolates of plant material, which included saponins, flavonoids, and anthocyanins, from *C. pentagyna*, demonstrated antiarrhythmic activity. Reports of past investigations of other species of *Crataegus* demonstrated pharmacological activity in the flowers, leaves, and fruit (7). The present study showed that the bark also possesses active principles.

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ACKNOWLEDGMENTS AND ADDRESSES

Received February 11, 1974, from the *Department of Pharmacognosy and Pharmacology, College of Pharmacy, University of Illinois at the Medical Center, Chicago, IL 60612*

Accepted for publication June 21, 1974.

The authors express their appreciation to Mr. Floyd Swink, Morton Aboretum, and Dr. Frank Crane, Director, University of Illinois Experimental Station, for the verification, collection, and supply of the plant material used in these studies.

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