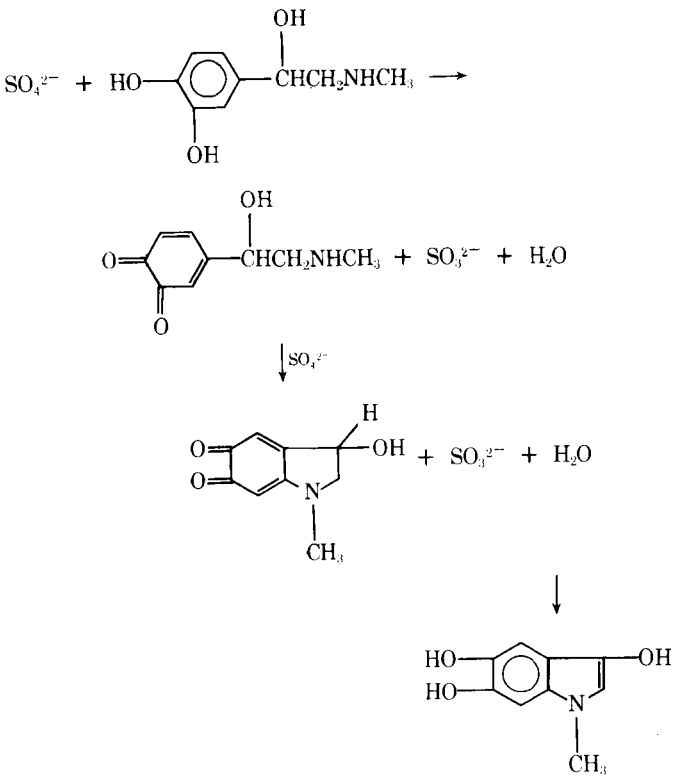

Scheme II

Previous success (10) in stabilizing different water solutions of epinephrine up to 5 years by preparing them in a nitrogen atmosphere confirms this hypothesis.

The results of the present experiments obtained in the nitrogen atmosphere show that oxygen in the reacting mixture is the most decisive factor for the stimulation of epinephrine oxidation in the presence of sodium metabisulfite in the given concentrations. Namely, the stimulating action of sodium metabisulfite on epinephrine oxidation is absent when dissolved oxygen from the air is eliminated from the solution of the reactant and when the reacting process goes on in the nitrogen atmosphere. In a highly alkaline medium, this process does not occur because of the strong influence of the hydroxide ions on epinephrine oxidation (Fig. 5).

Under the same experimental conditions, numerous experiments were performed using cysteine in different concentrations instead of sodium metabisulfite. Cysteine concentrations greater than 7×10^{-5} *M* efficiently inhibited epinephrine oxidation (Fig. 6). Since cysteine is an integral part of the human organism and since it maintains a kind of balance with the epinephrine concentration (11), it would be worthwhile to study its application in the stabilization of aqueous epinephrine solutions.

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Cardiovascular Effects of *Azadirachta indica* Extract

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Abstract □ A crude extract of the leaves of *Azadirachta indica* was studied for its effects on the cardiovascular system of anesthetized guinea pigs and rabbits. These effects include profound hypotension and a minimal negative chronotropic effect, which increased at higher doses. In one rabbit, 200 mg of extract/kg decreased the heart rate from 280 to 150 beats/min. The extract also exhibited a weak antiarrhythmic activity in rabbits against ouabain-induced dysrhythmia.

Keyphrases □ *Azadirachta indica*—extract of leaves, cardiovascular effects evaluated in guinea pigs and rabbits □ Cardiovascular effects—*Azadirachta indica* extract of leaves, evaluated in guinea pigs and rabbits

There is scanty literature information concerning the pharmacological properties of *Azadirachta indica*, an evergreen flowering plant belonging to the family Meliaceae. In Nigeria, this plant, called "dongoyaro," is used primarily to treat malaria, fever, abdominal disorders, and hemorrhoids and as an anthelmintic (1). Recent studies *in vivo* (2) failed to confirm the antimalarial effects attributed to this plant.

A preliminary pharmacological study (3) revealed that the extract acted as a spasmogen on isolated guinea pig ileum. It also increased the respiratory rate in the dog without a change in depth.

The present experiments were undertaken to elucidate further the pharmacological effects of an extract of the leaves of *A. indica* on the heart and circulatory system.

EXPERIMENTAL

Preparation of Crude Aqueous Extract—Air-dried powdered leaves[1] (1.8 kg) of *A. indica* were extracted with 3 liters of distilled water in a soxhlet apparatus for 1 week. The extract volume was then reduced to about 200 ml on low heat with constant stirring. The final 200 ml of

[1] The leaves were collected from plants on the University of Ife campus, Ile-Ife, Nigeria, during January. The plant material was identified as *A. indica* A. Juss. (Meliaceae) by Dr. E. A. Sofowora, Department of Pharmacognosy Incorporating Drug Research Unit, University of Ife, Ile-Ife, Nigeria, and authenticated by comparison with specimens in the Institute of Forest Research Herbarium, Ibadan, Nigeria. Voucher (preserved) specimens (No. UNIFE 426) are on deposit in the Museum and Herbarium of Nigerian Medicinal Plants of the University of Ife.

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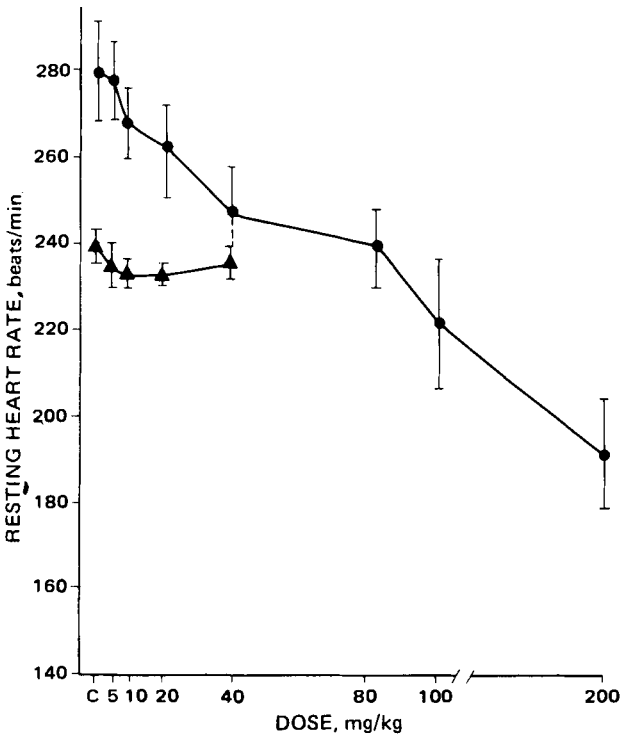

Figure 1—*Effect of* A. indica *extract on the spontaneous resting heart rate. Key:* ●, *rabbit response* (n = 3); *and* ▲, *guinea pig response* (n = 3).

brown extract was frozen and lyophilized to yield 250 g of a dark-brown powder, which was used for testing. Solutions (weight per volume) of this extract were freshly prepared in distilled water on the day of each experiment.

Preparation of Animals—Rabbits and guinea pigs of either sex, 1.0–2.2 kg and 200–250 g, respectively, were surgically prepared under urethan anesthesia (1000 mg/kg ip) for recording arterial blood pressure from a femoral artery and spontaneous heart rate and for intravenous drug administration by conventional methods (4). Only rabbits were tested for a 15-sec bilateral carotid occlusion response. This test involves the application of two bulldog clamps, one to each common carotid artery, to stop blood flow to the brain for 15 sec.

The cardiovascular effects of increasing doses of an aqueous extract of the leaves of *A. indica* were determined in three rabbits and three guinea pigs. Doses of 5, 10, 20, 40, 80, 100, and 200 mg/kg iv were tested. Each dose was administered at 30-min intervals while recordings of heart rate and arterial blood pressure were taken every 5 min. The antiarrhythmic potential of the extract also was determined in four other rabbits against ouabain-induced dysrhythmia according to a previously described method (5). The dose of the extract required to reverse sustained arrhythmia to normal sinus rhythm was determined.

RESULTS

Effect on Heart Rate—Figure 1 shows that increasing doses of the *A. indica* leaf extract progressively reduced the spontaneous heart rate of anesthetized rabbits. A dose of 200 mg of extract/kg greatly reduced the heart rate from both predrug and postdrug levels in these animals.

In anesthetized guinea pigs, the extract had no appreciable effect on the spontaneous heart rate, since the rate remained almost constant, varying only by two or three beats/min.

Effect on Blood Pressure—An extract of *A. indica* decreased arterial blood pressure (Fig. 2) but had no effect on the bilateral carotid occlusion response in anesthetized rabbits. The hypotensive effect of the extract was dose related and was much greater on diastolic than systolic pressure. Each dose level of the extract increased the arterial pulse width in these animals while it decreased the pulse rate.

In anesthetized guinea pigs, 5 and 10 mg of extract/kg transitorily increased arterial blood pressure; higher doses lowered the pressure. None of the three guinea pigs tested tolerated doses of the extract above 40 mg/kg without manifestations of toxicity and eventually death.

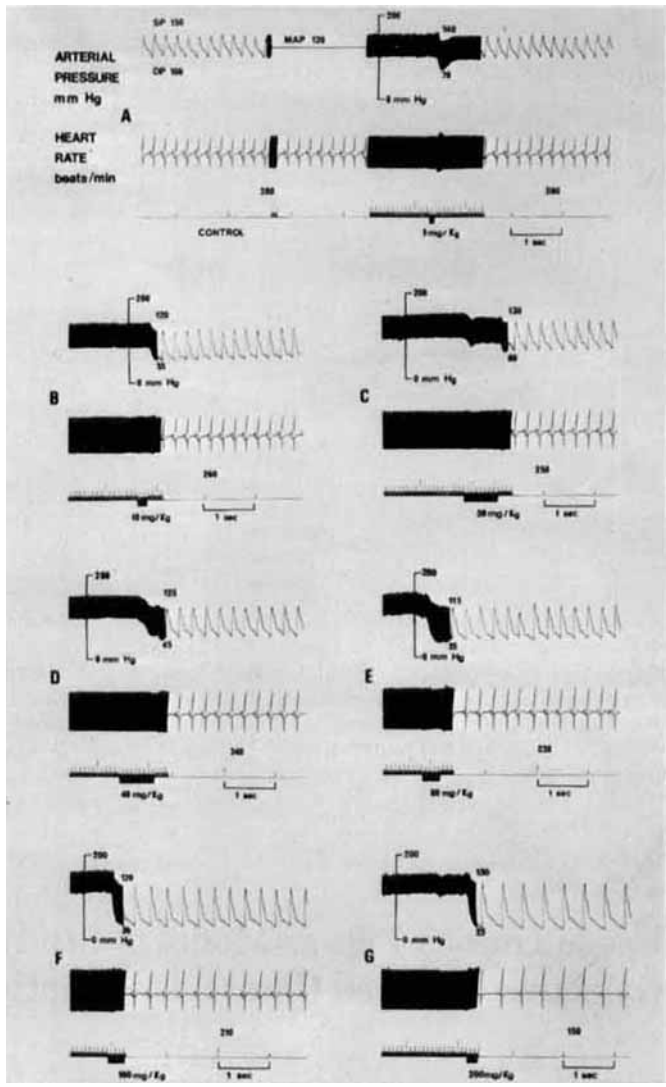

Figure 2—*Arterial blood pressure and heart rate response to* A. indica *extract. Key: A, control before drug administration; and B–G, responses to increasing doses of the drug.*

Effect on Ouabain[2]-Induced Cardiac Dysrhythmias—The sustained cardiac dysrhythmia induced by the serially administered doses of ouabain was reversed to normal sinus rhythm by 40 mg of extract/kg given in two equally divided doses. Normal rhythm returned within 8 min after the administration of the second dose (Fig. 3). It also decreased tachycardia from 380 beats/min, associated with the multifocal ventricular extrasystoles, to 220 beats/min.

DISCUSSION

In anesthetized guinea pigs and rabbits, an extract of the leaves of *A. indica* exerted some effects on the cardiovascular system. All doses tested had hypotensive effects. Reduction in arterial blood pressure was dose related. However, in anesthetized guinea pigs, small doses (5 and 10 mg/kg) induced a transient blood pressure rise. Since this effect was not observed in anesthetized rabbits, this difference seems to be species related.

The rise in arterial blood pressure observed in guinea pigs caused by low doses of the extract supports the suggestion (3) that the extract may have a biphasic effect on arterial blood pressure. In the anesthetized dog, intravenous administration of the extract produced an initial rise in arterial blood pressure, followed by a protracted fall below the normal level (3). In both species, the fall in arterial blood pressure was accompanied by an increase in the pulse width. This observation also confirms previous findings and suggests that the prolonged fall in blood pressure was due to the effect of the extract on vascular smooth muscle, probably giving

[2] Ouabain injection USP, Eli Lilly and Co.

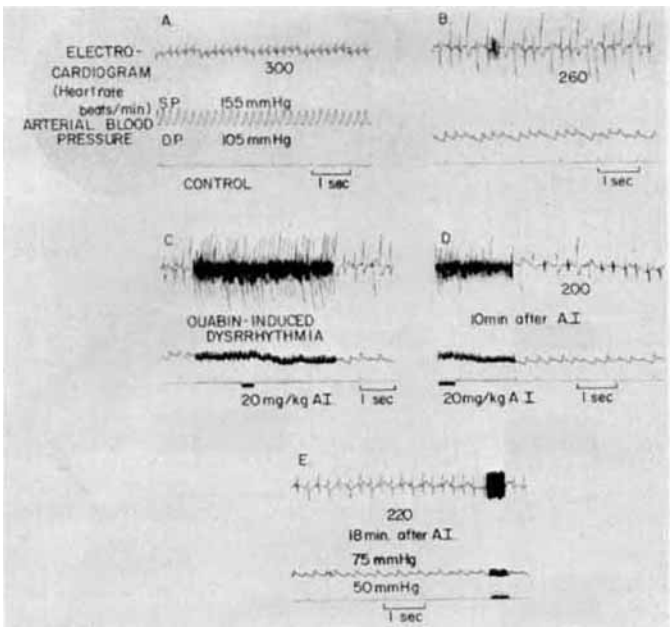

Figure 3—*Antiarrhythmic action of* A. indica *extract. Key: A, control before ouabain-induced dysrhythmia; B, 10 min after sustained dysrhythmia; C, after first dose (20 mg/kg) of extract; D, after second dose (20 mg/kg) of extract; and E, normal sinus rhythm 18 min after initial dose of extract.*

rise to a somewhat persistent vasodilation due to a reduction in arteriolar tone.

Heart rate remained nearly constant in the guinea pig, whereas there was a slight but progressive decrease in the rabbit. A direct depressant action of the compound is an untenable explanation of the mechanism of cardiac slowing in view of the observed increases in both pulse width and ECG amplitude, which may be evidence of a positive inotropic effect.

The extract of *A. indica* possessed weak antiarrhythmic activity against ouabain-induced cardiac dysrhythmias. The present experiments did not provide a satisfactory explanation of the mechanism involved, but the negative chronotropic effect of the drug appears to be beneficial in this respect.

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Use of Guinea Pigs as Model to Study Galactose-Induced Cataract Formation

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Received December 5, 1977, from the *Department of Pharmacology, Massachusetts College of Pharmacy, Boston, MA 02115.* Accepted for publication February 10, 1978.

Abstract □ Because the dietary requirement for ascorbic acid is similar in humans and guinea pigs, galactose-induced cataract research with the guinea pig as an experimental model instead of the rat might be appropriate and may represent a closer analogy to galactosemic cataract formation in humans. In this study, dietary ascorbic acid was found in all guinea pigs to have a retarding or delaying effect on the development of galactose-induced cataracts.

Keyphrases □ Models, experimental—guinea pig for study of galactose-induced cataract formation, effect of ascorbic acid □ Galactose-induced cataract formation—studied in guinea pigs, effect of ascorbic acid □ Ascorbic acid—effect on galactose-induced cataract formation in guinea pigs

Galactose fed in high enough concentration causes cataracts in experimental animals (1–3). In humans, cataract formation is a prevalent complication of galactosemia, an inborn error of metabolism associated with impaired galactose biotransformation.

BACKGROUND

Classic galactosemia results from a deficiency of the enzyme galactose 1-phosphate uridyl transferase, which leads to tissue accumulation of galactose 1-phosphate and galactose. Once inside the lens, galactose is reduced by aldol reductase to galactitol, to which the lens is impermeable. A high concentration of galactitol within the lens of the galactosemic rat leads to an osmotic overhydration, which ultimately causes the formation of mature cataracts (4–6).

The early observation that scorbutic guinea pigs showed a decline in lens ascorbic acid content led to the production of cataracts in a small percentage of guinea pigs by feeding them a scorbutic diet (7). Galactose cataracts also developed in guinea pigs maintained on a scorbutic diet that included 30% casein (8).

There is a scarcity of literature on the use of the guinea pig as an animal model for the study of galactose cataracts, but a large number of studies used the rat. Of particular importance in this regard is the renewed interest in the use of flavonoids (9) or vitamin diets (10) to delay the formation of galactose-induced cataracts in the rat.

Unlike the rat, however, humans and guinea pigs cannot synthesize their own daily requirements of ascorbic acid, which is essential in the daily diet to prevent scurvy. Because of this similarity and because of the infrequent use of the guinea pig as an experimental model for the study of galactosemic cataracts, the present study was undertaken to determine if ascorbic acid could effectively delay the onset of galactosemic cataracts in the guinea pig.

EXPERIMENTAL

Fifteen Hartley strain guinea pigs, approximately 300 g, were housed in three gang cages, five per cage. The dietary protocol is shown in Table

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