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CARDIOVASCULAR EFFECT OF *ARTEMISIA AFRA* AND ITS CONSTITUENTS

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ABSTRACT

The cardiovascular effects of a mixture of long chain fatty esters ($C_{44}H_{88}O_2$) and scopoletin isolated from Atemisia afra *and an aqueous extract of the plant were investigated in rabbits. The long chain fatty esters induced hypotensive effects at doses of 0.5, 1.0, 1.5 and 3 mg/kg. The diastolic pressure was affected more than the systolic. Aqueous* A. afra *extract (10–45 mg/kg) had a hypotensive effect* in vivo *and a dose-dependent biphasic effect on the heart* in vitro. *Lower doses induced an initial cardiostimulation followed by cardiodepression, whereas higher doses were mainly cardiodepressant. Scopoletin, a coumarin derivative, at a dose of 1.0–2.5 mg, induced a dose-dependent decrease in inotropic activity plus an appreciable decrease in chronotropic effects, especially at higher dose levels. These results suggest that* A. afra *and its constituents are potentially useful for the management of hypertensive conditions.*

INTRODUCTION

The genus *Artemisia* (Compositae) consists of several species widely used in many parts of the world either alone or in combination with other plants as herbal remedies for a variety of diseases including malaria (Klayman et al., 1984), tumors (Jeremic et al., 1973), and cardiovascular disorders (Ojewole & Adesina, 1983; Yamahara et al., 1989). *Artemisia afra*, found growing in South and East Africa including Kenya, has been used for malaria and other fevers, diabetes, and a wide range of other diseases (Watt & Breyer-Brandwijk, 1962).

This paper reports the results of cardiovascular studies performed with the aqueous extract of *A. afra* and a mixture of long chain fatty acylesters isolated from the petrol extract of the leaves of the plant, and scopoletin isolated from a methanol extract.

Keywords: Artemisia afra, long chain esters, aqueous extract, cardiovascular properties, hypotensive effect, cardiodepression.

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MATERIALS AND METHODS

Plant Material

The aerial parts of *Artemisia afra* were collected in Limuru near the Kinungi East and West road junction on the Nairobi/Naivasha road and also in Nakuru on the slopes of Menengai Crater. A small amount of the plant was also collected from Ngoina Hills in Sotik (Kericho) along the road.

The authenticity of the plant was in all cases established by the East African Herbarium, Nairobi, Kenya and voucher specimens are deposited at the Herbarium and at the Faculty of Pharmacy, University of Nairobi, Kenya.

Isolation of Fatty Acyl Esters (AAGl) from *A. afra* Leaves

The ground leaf powder of the plant was exhaustively extracted in a Soxhlet extractor with petrol (bp 60–80°C). The petrol extract was vacuum dried to give a dark green mass 120 g. This was triturated several times with ethanol (5 × 300 ml) and re-crystallised twice from warm ethanol. The ethanol insoluble solid AAG1 (m.p. 72–73°C; decomp) was characterised as a mixture of three long chain fatty acyl esters [($C_{44}H_{88}O_2$ (648), $C_{46}H_{92}O_2$ (676)] and $C_{48}H_{96}O_2$ (704) using

infra-red spectroscopy, chemical ionisation mass spectrometry and nuclear magnetic resonance spectrometry (Guantai, 1990).

Isolation of Scopoletin from *A. afra* Leaves

The petroleum ether exhausted material was Soxhlet extracted using 70% aqueous methanol for 48 h. The resulting extract was vacuum dried until all the methanol was removed. The remaining aqueous extract was sequentially solvent;solvent extracted using 8 × 300 ml portions of diethyl ether, ethylacetate and chloroform. Upon vacuum evaporation, the diethyl ether solid was triturated in methanol and the methanol soluble fraction was reduced to dryness. The resulting solid (57.22 g) was column chromatographed through 70 g silica gel (70–230 mesh ASTM-Merck). The column was eluted with chloroform:methanol (20:1) and the eluents monitored using thin-layer chromatography (polygram pre-coated silica gel plates – 0.25 mm thick with fluorescent indicator UV254; developing solvent chloroform:methanol, 10:1). Ultraviolet light and anisaldehyde spray reagent (Stahl, 1967) were used for detection. Sample one (fractions 1–96) showed very intense blue fluorescence and upon vacuum reduction yielded a solid that was taken up in a minimum amount of ethyl acetate and left to stand in the refrigerator at 4°C. Dirty yellow crystals were deposited which were recrystallised twice from a minimum volume of ethyl acetate to yield amber colored crystals (melting point 198–200°C). The crystals were confirmed to be scopoletin using spectrometirc methods (ultraviolet, infrared, proton and ^{13}C NMR, and electron impact mass spectrometry) (Guantai, 1990). The results were compared with those obtained using an authentic sample of scopoletin.

Cardiovascular Studies

Blood pressure was monitored using a Hellige GMBH (Freiburg) model SM 102 blood pressure transducer. Contractile responses on the mammalian isolated heart were recorded using a transducer attached to a Washington 400 MD ZC Oscillograph (Bioscience Sheerness, Kent, UK) at a chart speed of 0.25 mm/sec.

New Zealand white rabbits were anaesthetised with 25% urethane (7 ml/kg). The carotid artery and femoral vein were cannulated and a tracheal tube to ensure adequate ventilation was inserted into the trachea.

A 10 mg/ml suspension of the ester mixture (AAG1) in 2% acacia was prepared and administered intramuscularly at dose levels of 0.5, 1.0, 1.5 and 3.0 mg/kg via the thigh muscle. Controls were also set up using 0.3 ml of 2% acacia which was the highest volume of the diluent administered in the experimental animals.

The aqueous *A. afra* extract was prepared by boiling 20 g of the powdered leaf for at least 30 min. The contents were filtered while hot and the residue was exhaustively washed with hot water. The filtrate plus the washings was freeze-dried to a solid A,W. which was subsequently screened for cardiovascular activity after reconstitution with water.

Doses ranging from 10–45 mg/kg were administered slowly through the femoral vein. Blood pressure changes were recorded every 15 min.

Appropriate controls were also set up using water. All readings were expressed as percentages of the baseline values.

RESULTS AND DISCUSSION

The aqueous *A. afra* extract induced a rapid onset of action (5 min) and a long duration (90 min) after i.v. administration. At the dose levels tested, the aqueous *A. afra* extract induced a progressive fall in both systolic and diastolic blood pressure with maximum mean percent reduction values of 5.8, 14.3 and 31.2% systolic for 10, 20 and 45 mg/kg, respectively, being attained within the first half hour (Fig. 2).

There was gradual recovery with significant depressor effects being observed even after 90 min (14.5% systolic and 18.6% diastolic for the 45 mg/kg dose) (Figs. l and 2).

Intramuscular doses of AAG1 induced a slow onset but long duration of action. There was a progressive fall in both systolic and diastolic pressures with the overall reduction in diastolic pressure being more pronounced (Figs. 3 and 4). A dose of 0.5 mg/kg caused a reduction of systolic pressure by 3.0 and 23.0% within the first and 3rd h, whereas the diastolic pressure fell by 3.5 and 20.5%, respectively, within the same time interval. At twice the dose (1.0 mg/kg, the mean percent reduction in systolic pressure ranged from 7.8 within the first hour to 32.4% by the end of the 3rd h, while the corresponding figures for diastolic pressure were 6.4 and 44.6%, respectively (Figs. 3 and 4).

After the 1.5 mg/kg dose, the mean percent reduction in systolic pressure at the end of 1 h and 3 h was 6.1 and 34.6, respectively, while that of the diastolic pressure was 7.3 and 24.4% for the same dose and time interval. These results are not appreciably different from those obtained with a dose of 1.0 mg/kg. The difference between potency of 1.5 and 3.0 mg/kg within

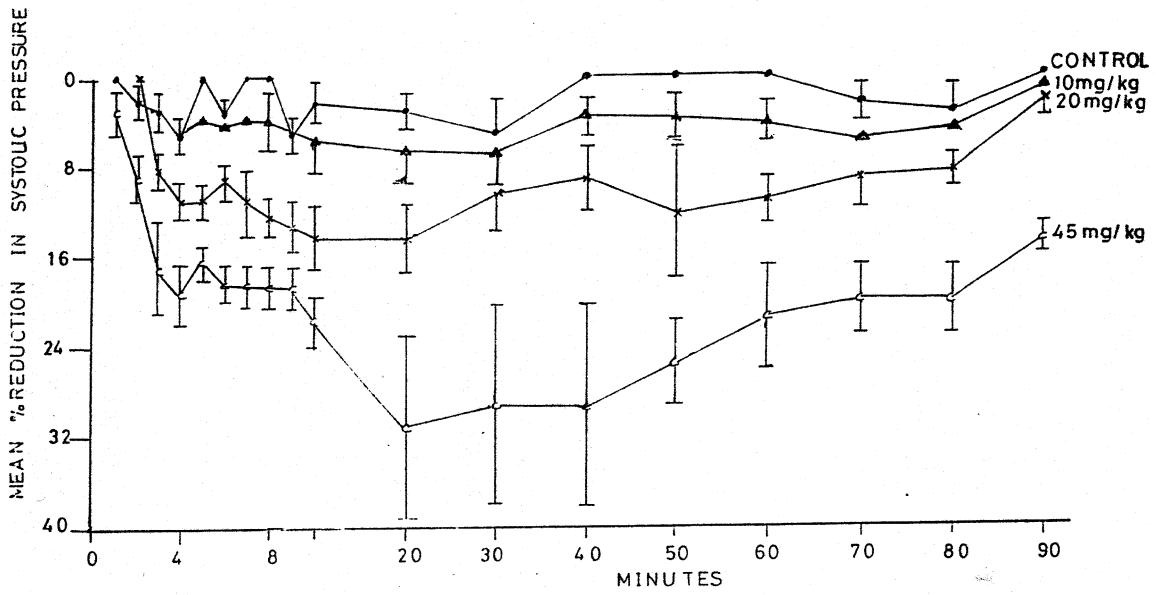

Fig. 1. Effect of intravenous injection of aqueous *A. afra* extract on the blood pressure of anaesthetised rabbit (systolic pressure). N = 6 Error bars = SEM.

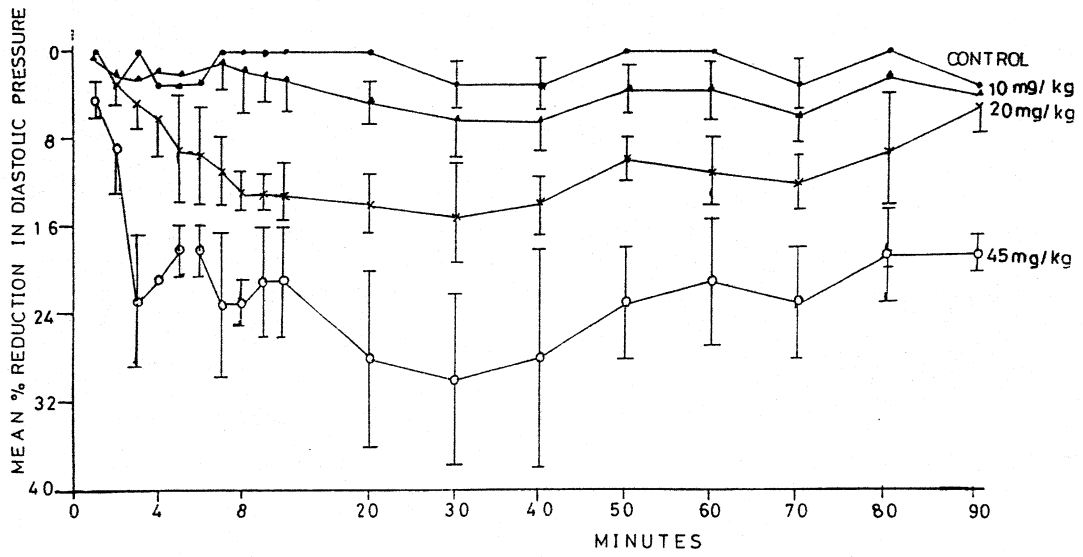

Fig. 2. Effect of intravenous injection of aqueous *A. afra* extract on the blood pressure of anaesthetised rabbit (diastolic pressure). N = 6, Error bars = SEM.

the first 2 h is also not evident. However, after 3 h, the 3.0 mg/kg dose induced a mean percent decrease in diastolic pressure more than 1.5-times greater than that induced by the lower doses.

The results do not show a clear dose-dependent response, but the prolonged hypotensive effect of both AAG1 and the aqueous *A. afra* extract is clear.

A dose of adrenaline (4 μg/kg, i.v.) induced a brief pressor effect (10.4% systolic and 13.7% diastolic) after which the hypotensive effect of AAG1 continued to be manifested. A similar response was observed ater co-administration of adrenaline (4 μg/kg) and aqueous *A. afra* extract (45 mg/kg).

From the results obtained with the long chain esters and aqueous *A. afra* extract, it can be concluded that the aqueous *A. afra* extract has a lower potency and a relatively shorter duration of action (Figs. 1, 2, 3 and 4). *Aqueous A. afra* extract and AAG1 have been shown to

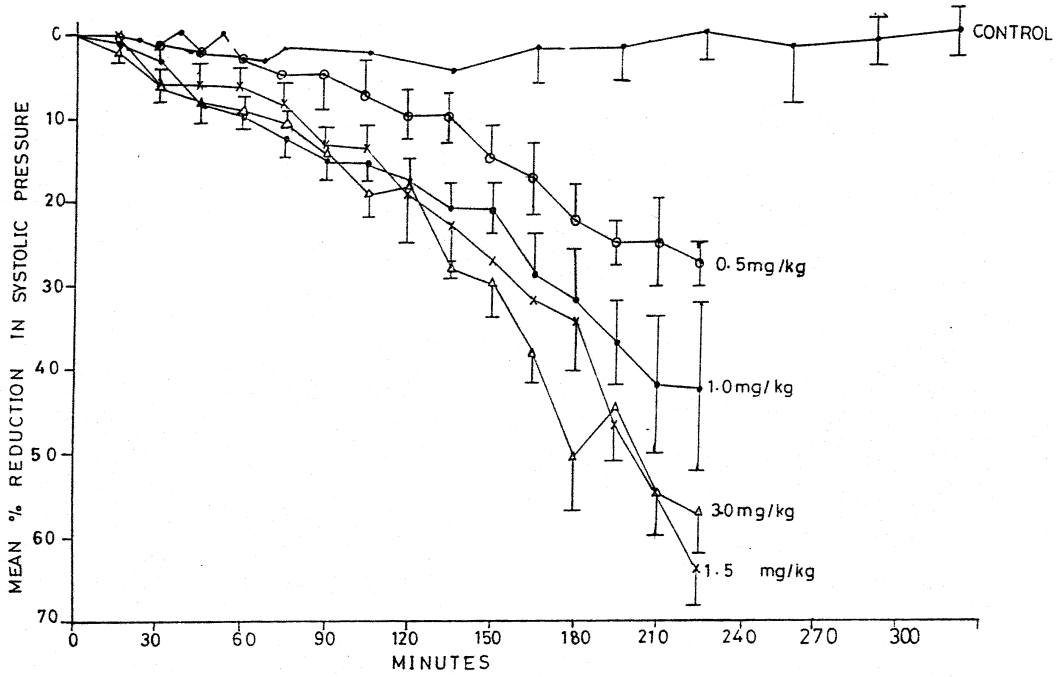

Fig. 3. Mean percent reduction is systolic pressure of an anaesthetised rabbit induced by intra-muscular doses of AAG1. N = 6, Error bars = SEM.

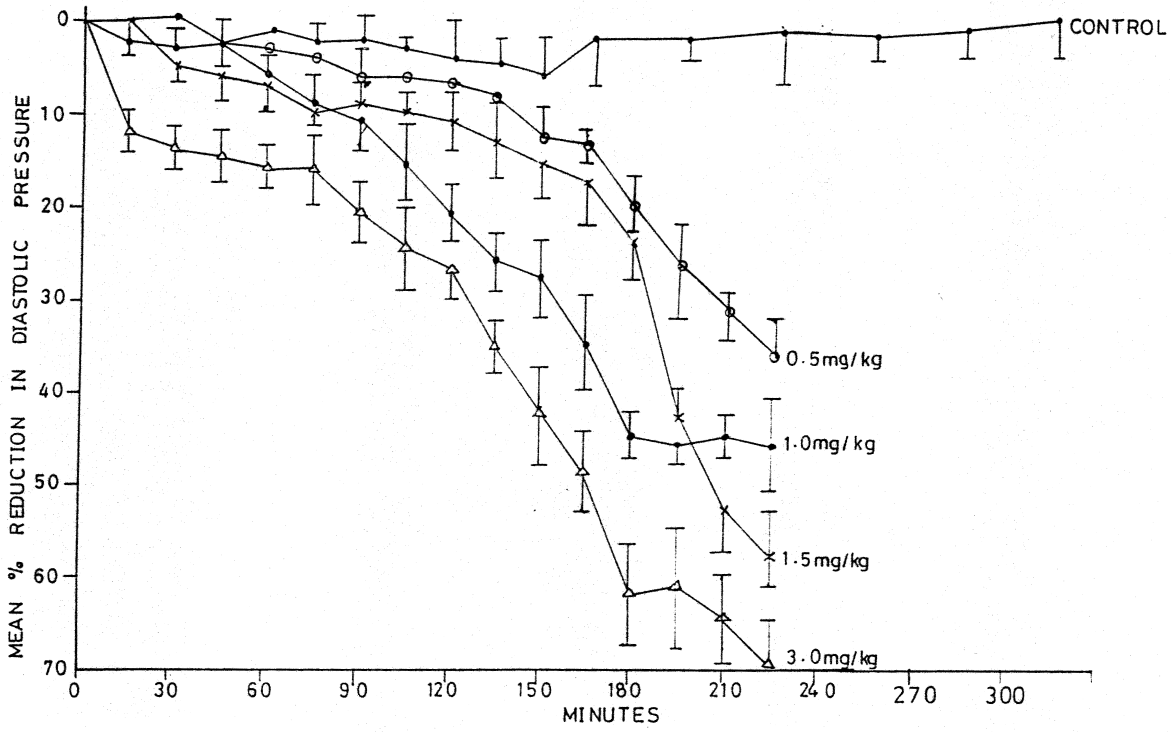

Fig. 4. Mean percent reduction in diastolic pressure of anaesthetised rabbit induced by intra-muscular doses of AAG1. N = 6, Error bars = SEM.

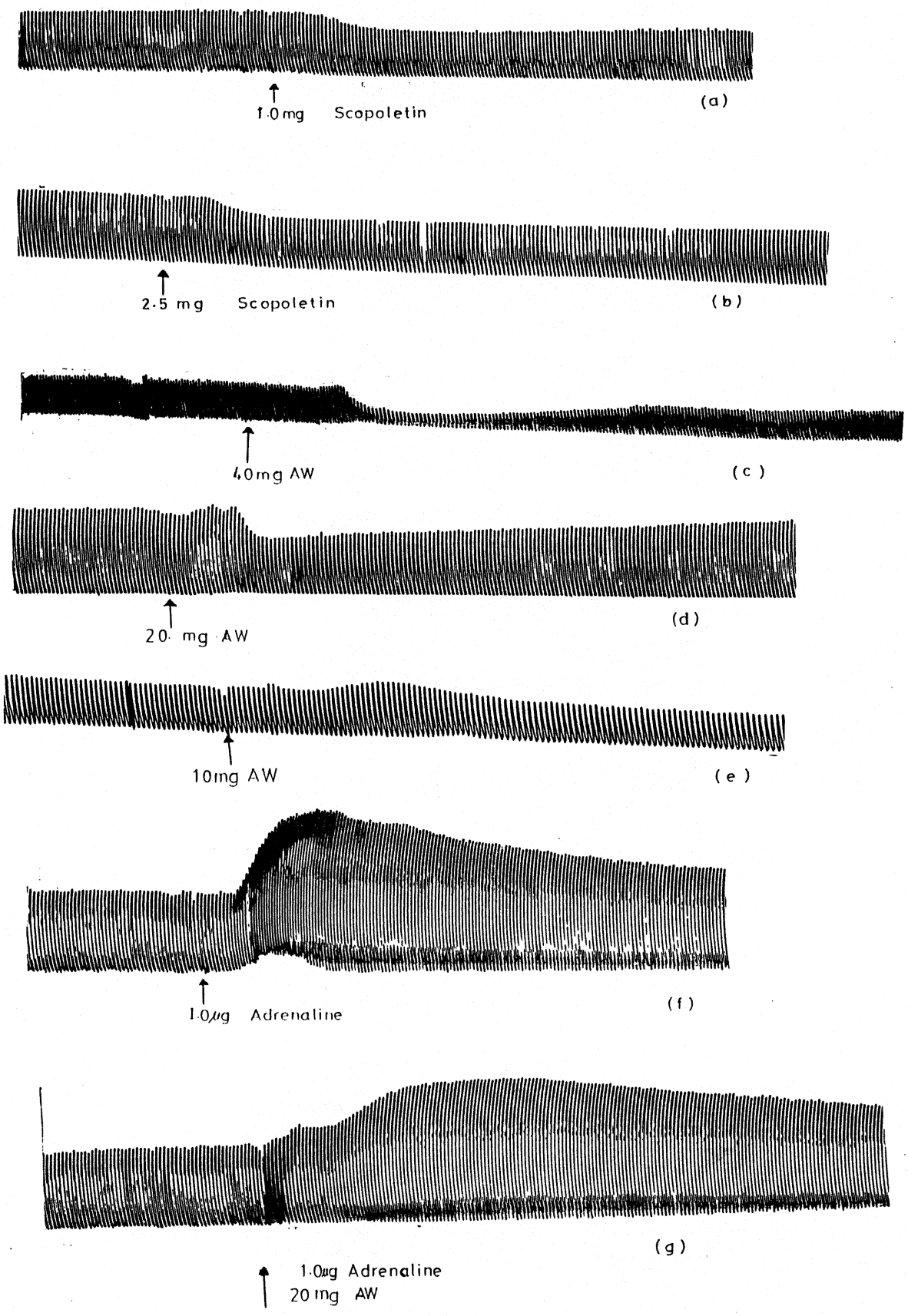

Fig. 5 (a-g). Effect of scopoletin and aqueous *A. afra* extract (AW) on the contractile activity of mammalian isolated heart.

be orally potent as depressor agents (Guantai, 1990). This coupled with the relatively long duration of action, hence fewer daily doses, makes the test compounds good candidates in the management of hypertension. The plant material has also been shown to be devoid of acute toxic effects (Guantai, 1990).

When tested on the rabbit isolated heart, the aqueous *A. afra* extract was found to have a biphasic effect. Low doses caused mainly a mild cardiostimulant effect (Fig. 5e) whereas higher doses caused an intial brief cardiostimulation followed by cardiodepression (Fig. 5d). Increasing the dose further led to mainly cardiodepressant effects (Fig. 5c).

Co-administration of adrenaline (1 μg) and 20 mg of the aqueous extract (Fig. 5f) caused a decrease in the inotropic effect of adrenaline 23.8%) with a significant decrease in the chronotropic effect induced by adrenaline also being manifested.

At 1 mg and 2.5 mg dose levels, scopoletin induced a dose-dependent decrease in inotropic activity (–22.2% and –33.3%, respectively) accompanied by an appreciable decrease in chronotropic effect, especially with the higher dose. The hypotensive effect of scopoletin (Ojewole & Adesina, 1983) could partly be as a result of the negative inotropic and chronotropic effects observed in the present study.

The dose-response variation in the cardiac response to the aqueous *A. afra* extract could be explained by the fact that the active substance(s) have a biphasic response on the heart, the overall effect being determined by the dose. Similar dose-related biphasic responses have previously been observed with other cardiovasccular agents such as ephedrine and amphetamine (Trendlenberg et al., 1963; Goldberg & Shideman, 1967; Guantai, 1982) and might have a bearing on the type of chemical transmitter that is released at the receptor sites upon drug receptor interaction (Goldberg & Shideman, 1967; Guantai, 1982).

The properties of *A. afra* and its constituents reported in this study are suggestive of potential antihypertensive agents that are inexpensive and locally available. However, more studies, especially with regard to chronic toxicities in animals, are currently being carried out in our laboratories before the agents can be claimed to be safe for clinical application.

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REFERENCES

Goldberg AM, Shidema FE (1967): The negative inotropic action of ephedrine and its modification by various agents. *Pharmacol Exp Therap 157* (1): 49–53.

Guantai AN (1982): Catha Edulis (Vahl) Forsk, Miraa, occurrence, active constituents and pharmacological activity. MSc. Thesis, University of Nairobi, Kenya.

Guantai AN (1990): Chemotherapy of malaria with special reference to drug interactions with chloroquine and herbal management of malaria in Kenya. Ph.D Thesis, University of Nairobi, Kenya.

Jeremic D, Jovic A, Behbud A, Stefanovic M (1973): Isolation of a potent anti-tumor agent arteanuin B from *Artemisia annua. Tetrahedron Lett 32*: 3039–3042.

Klayman DL, Lin J, Acton N, Scovill JP, Hoch JM, Milhous WK, Theoharides AD (1984): Isolation of artemisinin (Quinghaousu) from *Artemisia annua* growing in the United States. *J Nat Prod 47*: 715–717.

Ojewole JAO, Odesina SK (1983): Mechanism of the hypotensive effect of scopoletin isolated from the fruits of *Tetrapleura etraptra. Planta Med 49*: 46–50.

Stahl E (1967): *Dunnschicht Chromatographic* p. 817. Springer, Berlin 2nd edn.

Trendelenburg U, Alongo G, Del la Sierra, Muskus A (1967): Modification by reserpine of the response of the atrial pace-maker to sympathomimetic amines. *J Pharmacol Exp Ther 141*: 301–309.

Watt JM, Breyer-Brandwijk (1962): *The Medicinal and Poisonous Plants of Southern and Eastern Africa*. Second Edition. E & S Livingstone Ltd, Edinburgh and London, 514 pp.

Yamahara J, Kobayashi G, Matsuda H, Katayama T, Fujimura H (1989): The effect of scoparone, a coumarin derivative isolated from the Chinese crude drug *Artemisia capillaris* Flos on the heart. *Chem Pharm Bull 37*: 1297–1299.

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