THE MECHANISM OF HYPOTENSIVE EFFECT OF GARLIC EXTRACT

Pages with reference to book, From 357 To 362

Abdul Rashid (Department of Pharmacology, Ayub Medical College, Abbottabad.)
Hidayat Hussain Khan (Pharmacology Department.Postgraduate Medical Institute, Lahore.)

Abstract

Study was designed to elucidate the mechanism of hypotensive effect of garlic extract in dogs. The antagonists for common endogenous hypotensive substances (histamine, Acetylcholine, 5 Hydroxy Tryptamine Kinins and prostaglandins) were tried to confirm or exclude the possibility that garlic extract either acts like or causes release of any one of these endogenous hypotensive agents.
The results indicated a possible prostaglandin like effect of garlic extract.(JPMA 35 357, 1985).

INTRODUCTION

Ailium sativum (garlic) has been used as a food and remedy of various ailments for centuries. In the form of oils, liniments, poultices and powders, garlic has been extensively used in the Indo-Pak subcontinent, in indigenous system of medicine for treatment of hypertension, atherosclerosis, rheumatism, bronchial asthma, and as a spasmolytic, vermifuge and antiseptic.[1] Stoll and Seeback[2] isolated one of the active principles of garlic and named it as allin.

Bordia et al[3] claimed the protective action of garlic and onion in cholesterol fed rabbits. They have reported the hypolipaemic and increased fibrinolytic activity of garlic in fat produced alimentary lipaemia in volunteers. They deduced that the activity of garlic lies in an essential oil which is chemically a combination of sulphur containing compound (Allyl propyl disulphide, diallyl disulphide). Garlic increased the fibrinolytic activity and lowered the serum cholesterol by virtue of the essential oils of garlic. Malik and Siddiqui[4] demonstrated a hypotensive effect of garlic sap in dogs. They observed a significant fall in blood pressure lasting for 1-5 minutes, after intravenous administration of garlic sap in dogs. They also showed that garlic sap increased the contractions of isolated guinea-pig ileum and this action was completely blocked by dimetane (an anti-histamine).

Later Sia[5] demonstrated a partial blockade of the hypotensive effect of garlic sap by dirnetane, postulating that garlic acts by releasing histamine. Present work has been designed to evaluate the mechanism of the hypotensive effect of garlic extract.

MATERIAL AND METHODS

Thirty six dogs of either sex (weighing 10-15 Kg) were used for the study of hypotensive effect of garlic extract. They were divided into six groups, each comprising of six animals. Sixteen young virgin female rats were used for various experiments on rat uterus. Sixteen guinea-pigs of either sex were used for various experiments on guinea-pig ileum.

Garlic extract was prepared by the method of Sial.[6] Peeled, uncrushed cloves of garlic were extracted out thrice with absolute alcohol at an interval of 48 hours. The combined alcoholic extract was freed of solvent and residue was partitioned between ethylacetate and water. The water soluble portion was freed of solvent and the residue was divided up into alcohol soluble and alcohol insoluble portions. Water soluble but alcohol insoluble fraction was used in this study. The yield of this fraction was 0.8 percent. Stock solution was prepared in normal saline and kept at 4°C. Further dilutions were made

with normal saline before use.

Dogs were anaesthetized with pentobar. bitone sodium (30 mg/kg) intravenously[7]. Right femoral vein was cannulated and attached to a reservoir filled with normal saline. This route was used for administration of various solutions and drugs. Right common carotid artery was cannulated and attached to a pressure transducer (P-1000-B). Sodium citrate (9%) was used in the intervening tube separating blood from the pressure transducer. Blood pressure was recorded on the new 8 channel, Narcotrace 80 data acquisition system (Polygraph). Pressure coupler was calibrated in such a way that 5 cm rise of writing pen was equal to 100 mm of mercury. Percent changes in various components of blood pressure were calculated similar to Folle and Aviado[8]. The effect of garlic extract on blood pressure was recorded before and after pheniramine maleate, cimetidine HCI, pheniramine maleate plus cimetidine HC1, cyproheptadine HC1, flufenamic acid and atropine.

The procedure for isolated rat-uterus preparation was adopted as described in "Pharmacological experiments on isolated pfeparations[9] ". Each female rat was inj ected stilboesterol (0.1 mg/kg) subcutaneously in Order to induce oestrus, 24 hours before the rat was sacrificed. The effect of garlic extract on isolated rat uterus was studied before and after atropine, cyproheptadine HCI and flufenamic acid.

The procedure for isolated guinea-pig ileum preparation was adopted as described in "Pharmacological experiments on isolated preparations[9] ". Each guinea-pig was kept fasting for 24 hours before the animal was sacrificed, and then the effect of garlic extract was studied with and without pheniramine maleate, atropine, flufenarnic acid and cyproheptadine HCI, The effect of garlic extract on perfusion rate of rat-hind quarter preparation was also studied as mentioned by Sial[6].

RESULTS

Effect on Blood Pressure:

Garlic extract was used in the doses of 41.5, 83, 166, 332 and 664 mg/kg. This range was assessed by performing some pilot experiments. Garlic extract in a dose of 41.5 mg/kg produced a mean percent fall of 11.5, 23.99 and 19.87 in systolic, diastolic and mean diastolic blood pressures respectively. With increasing doses, the fall of blood pressure also increased, and 332 mg/kg of garlic extract produced a maximum mean percent fall of 32.76, 59.87 and 50.30 in systolic, diastolic and mean diastolic blood pressures respectively. This fall in blood pressure was statistically highly significant (Table I, Fig. I).

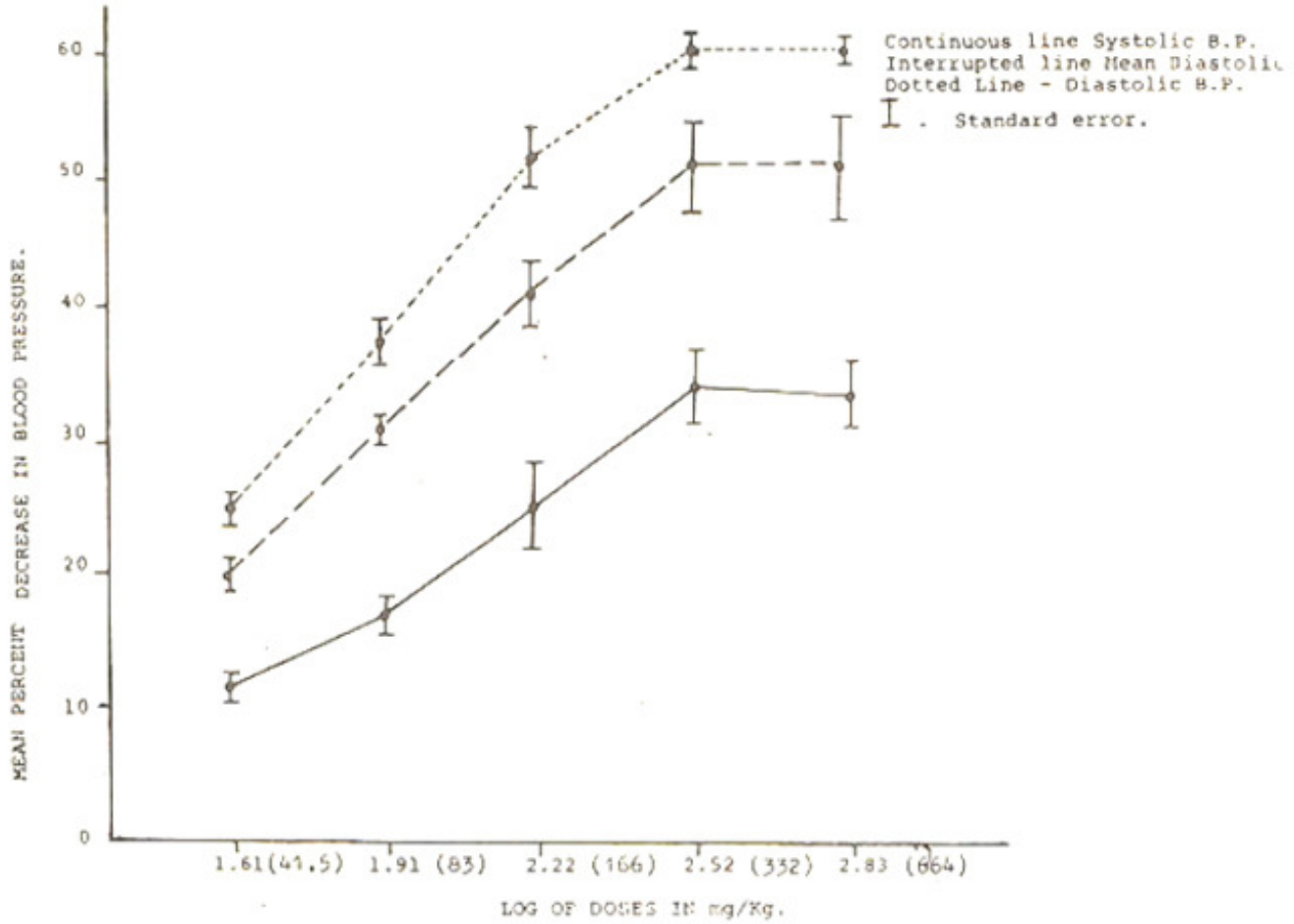

Fig.1. Log dose response curves showing the effect of garlic extract on blood pressure of dogs.

Table I
Mean Percent decrease in Blood Pressure by various Doses of Garlic Extract in Dogs.

Blood Pressure in mm Hg	Doses of Garlic Extract. 41.5 mg/kg	83 mg/kg	166 mg/kg	332mg/kg	664 mg/kg
Systolic	11.54 ± 1.81 (6)	15.13 ± 2.47 (6)	24.12 ± 3.28 (6)	32.76 ± 2.40 (6)	32.47 ± 2.38 (6)
Diastolic	23.99 ± 1.72 (6)	37.35 ± 2.75 (6)	50.90 ± 1.69 (6)	59.87 ± 0.85 (6)	59.68 ± 0.94 (6)
Mean B.P.	19.87 ± 1.99 (6)	30.86 ± 2.46 (6)	40.44 ± 2.66 (6)	50.30 ± 3.31 (6)	49.90 ± 3.36 (6)

Figures in parenthesis indicate number of animals.

The fall in blood pressure started after a mean of 5.01 (S.E±0.0l) seconds of garlic extract administration and reached maximum :in a meantime of 16.92 (S.E.±0.48) seconds. Time required for

complete recovery was a mean of 3.19. (S.E.±0.26) minutes.
Garlic extract in a dose of 166 mg/kg produced 75 percent of maximum response and was thereafter used for investigating the mechanism of hypotensive effect of garlic extract. The hypotensive effect of garlic extract was not antagonized by pheniramine (4 mg/kg), cimetidine (0.1 mg/kg), plus Pheniramine (4 mg/kg), cyproheptadine (20 mg/kg), flufenamic acid (20 mg/kg) and atropine (0.1 mg/kg) (Table-II).

Table II

Mean Percent Fall in Blood Pressure before and after Pheniramine maleate, Cimetidine HCl, Pheniramine maleate plus Cimetidine HCl, Cyproheptadine HCl and Flufenamic Acid by Garlic Extract (166 mg/kg) in Dogs.

Blood Pressure	Garlic Extract only	Mean per cent fall after Pheniramine maleate and Garlic Extract.	Garlic Extract only	Mean per cent fall after Cimetidine HCl and Garlic Extract	Garlic Extract only	Mean per cent fall after Pheniramine maleate plus Cimetidine HCl and Garlic Extract	Garlic Extract only	Mean per cent fall after Cyproheptadine HCl	Garlic Extract only	Mean per cent fall after Flufenamic acid	Garlic Extract only	Mean per cent fall after Atropine
Systolic	24.12±3.28 (6)	23.7±2.55 (6)	23.04±1.27 (6)	23.85±1.23 (6)	23.97±2.63 (6)	24.10±2.91 (6)	24.10±2.91 (6)	23.04±1.27 (6)	24.84±2.88 (6)	21.63±2.69 (6)	14.53±1.73 (6)	14.28±1.91 (6)
Diastolic	50.90±1.69 (6)	52.83±3.17 (6)	34.19±2.84 (6)	33.92±2.88 (6)	29.50±2.17 (6)	29.05±2.08 (6)	34.19±2.84 (6)	33.92±2.86 (6)	25.90±4.06 (6)	25.14±4.12 (6)	25.33±2.99 (6)	25.79±2.50 (6)
Mean B.P	40.44±2.66 (6)	41.6±2.81 (6)	29.44±2.01 (6)	29.38±1.89 (6)	27.17±1.75 (6)	27.39±1.74 (6)	29.44±2.01 (6)	29.38±1.89 (6)	25.25±2.06 (6)	23.45±2.28 (6)	20.00±2.57 (6)	20.20±2.24 (6)

Figures in parenthesis indicate number of animals.

Effect on Rat uterus

Garlic extract in the doses of 100,200,400 and 800 mg/25 ml produced contractions of rat uterus (Table-III).

Table III
Effect of different Doses of Garlic Extract on Rat Uterus.

No. of Exp.	Amplitudes of contractions in mm. Garlic Extract in mg/25 ml.				
	100	200	400	800	1600
1.	4	7	11	15	15
2.	5	6	9	14	14
3.	4	5	10	16	16
4.	3	7	11	17	17
Mean	4.00	6.25	10.25	15.40	15.40
S.E.	±0.40	±0.47	±0.46	±0.61	±0.61
P. Value	<0.001	<0.001	<0.001	<0.001	<0.01

This effect was significantly blocked by flufenamic acid (0.2 mg/25 ml) (Fig.2).

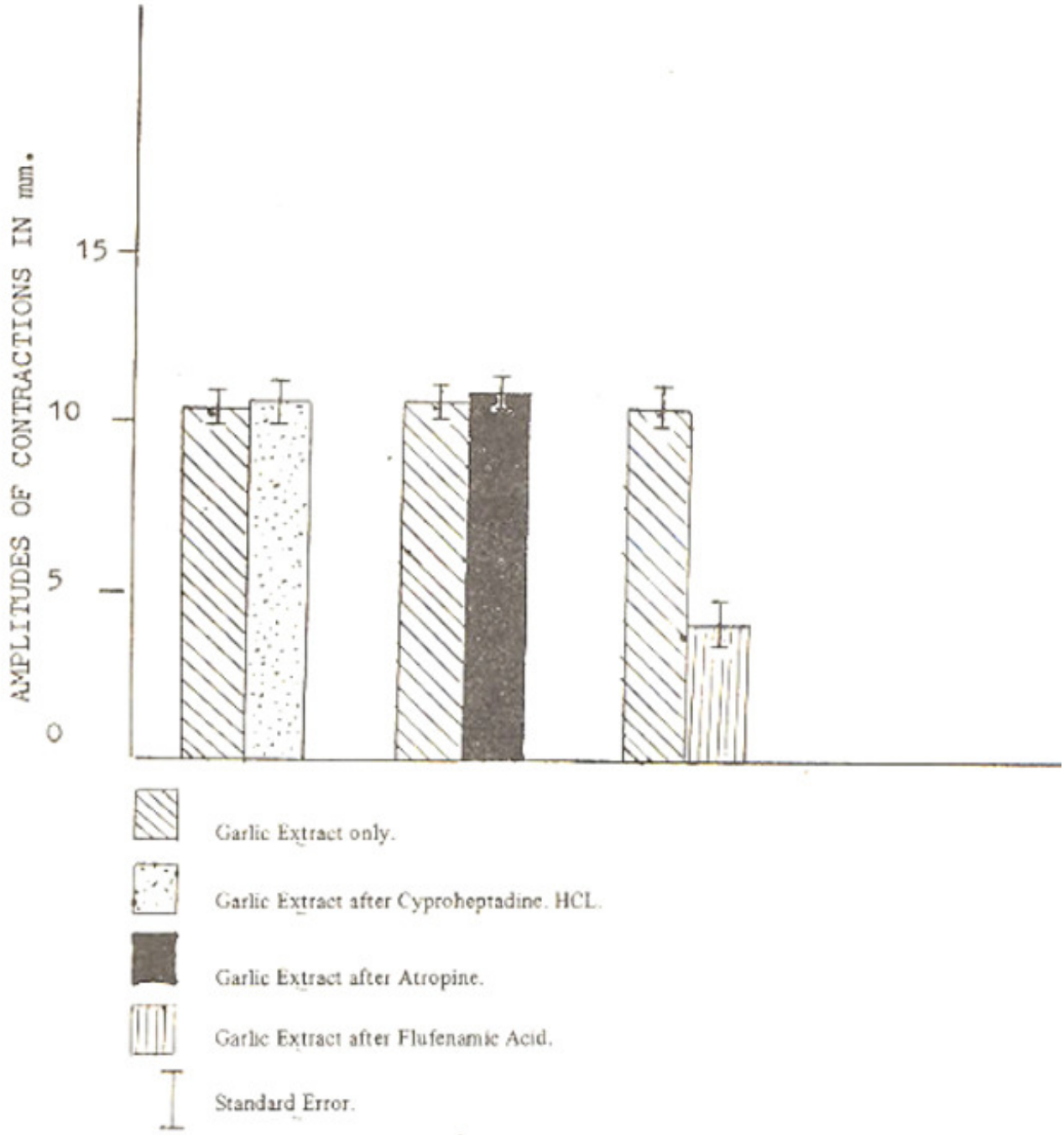

Fig. 2. Effect of garlic extract (400 mg/25 ml) on rat uterus before and after different antagonists.

Cyproheptadine and atropine failed to block the oxytocic effect of garlic extract.

Effect on Guinea. Pig, Ileum

Garlic extract in the doses of 0.4, 0.8, 1.6 and 3.2 mg/25 ml produced contractions of guinea.pig ileum (Table IV).

Table IV

Effect of different Doses of Garlic Extract on Guinea-Pig Ileum.

No. of Exp.	Amplitudes of Contractions in mm. Dose of Garlic Extract in mg/25 ml.				
	0.4 mg	0.8	1.6	3.2	6.4
1.	6	10	12	19	19
2.	5	9	13	18	18
3.	7	9	14	20	20
4.	6	7	11	18	18
Mean	6.80	8.75	12.5	18.75	18.75
S.E.	±0.40	±0.62	±0.64	±0.47	±0.47
P. Value	<0.001	<0.001	<0.001	<0.001	<0.001

This effect was also significantly blocked by flufenamic acid (0.2 mg/ 25 ml) (Fig 3).

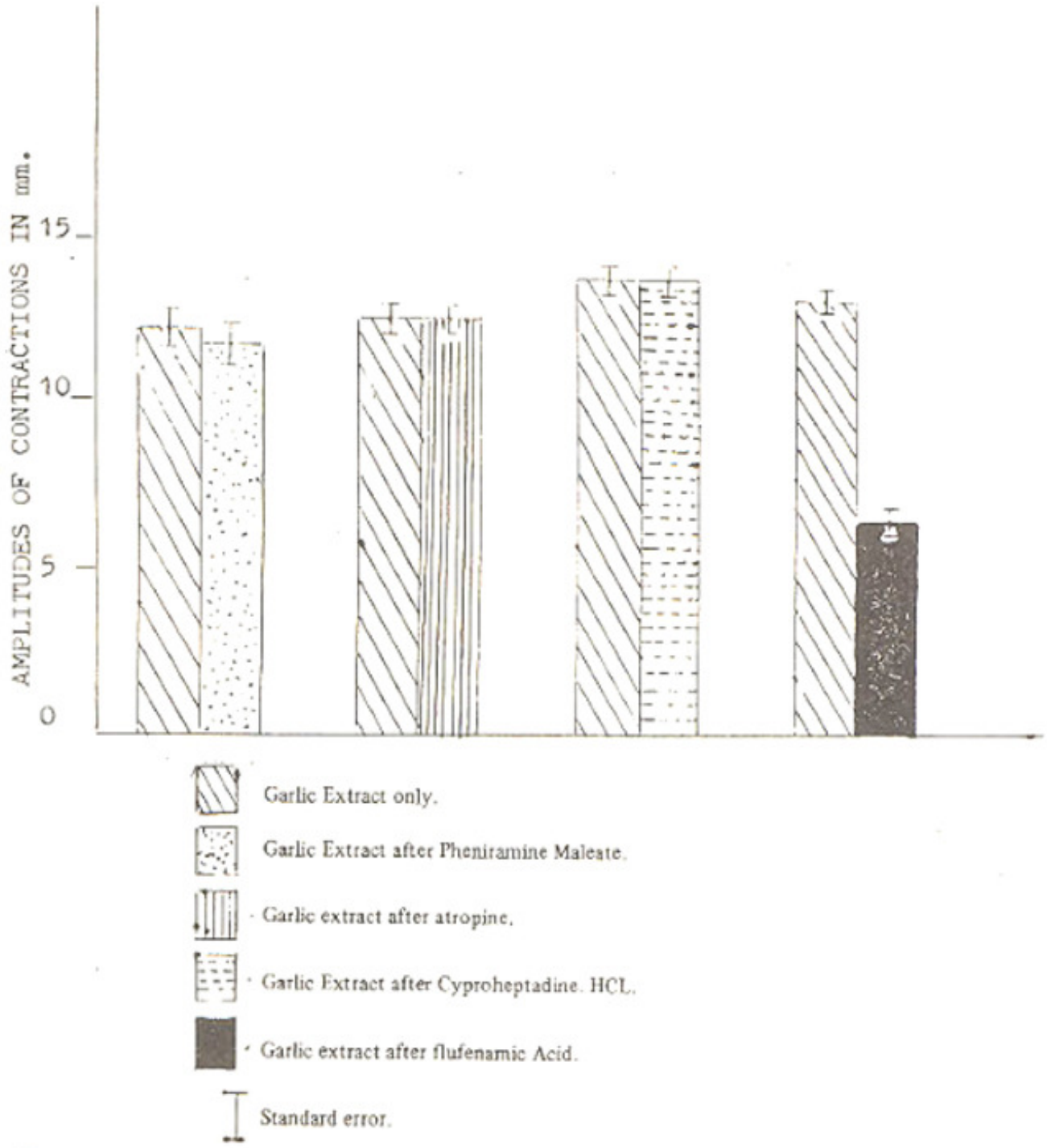

Fig. 3. Effect of garlic extract (1.6 mg/25 ml) on guinea-pig ileum before and after different antagonists.

The other antagonists like atropine, Cyproheptadine and pheniramine could not block the effect of garlic extract on guinea-pig ileum.

Effect of Garlic extract on perfusion rate of Rat-Hind Quarter

Garlic extract in the dose of 200 mg produced a significant increase in the perfusion rate of rat-hind quarter(Table V).

Table V

Effect of Garlic Extract on Volume of Fluid collected from Rat Hind-Quarter preparation in 5 Minutes.

No. of Expt.	Volume of Fluid in ml collected in 5 Minutes.	
	Before Garlic Extract	After Garlic Extract 200 mg.
1.	10.00	11.20
2.	8.00	9.50
3.	8.50	10.00
4.	9.00	10.20
Mean.	8.87	10.22
S.E.	±0.42	±0.35
P. Value	–	>0.05

DISCUSSION

Garlic extract produced a significant fall in systolic, diastolic and mean diastolic blood pressures of anaesthetized dogs. This effect of garlic extract indicates that one of the chemical constituents of garlic produces hypotension directly or indirectly. Sial[6] after performing cross circulation experiments on dogs, deduced that garlic extract does not act centrally, i.e. on vasomotor centre. He postulated that hypotensive effect of garlic extract was due to release of histamine, as he was able to block this effect by dimetane maleate (H1-receptor blocking antihistamine) partially but significantly. In this study, however, we could not observe any antagonism of the hypotensive effect by pheniramine maleate (H_1 -receptor blocking agent). Hence our findings are not in accordance with those of Sial. The hypotension produced by histamine is due to its action on both H_1 and H_2 -receptors which are present in blood vessels[10] In our experiments, both H_1 receptor and H_2 -receptor blocking agents, administered together,

were unable to prevent the hypotensive effect of garlic extract. In the light of this observation, it is not probable that garlic extract acts by releasing histamine.

The release of common endogenous hypotensive substances (histamine, acetylcholine, serotonin and kinins) by garlic extract or its action through a similar mechanism was excluded by prior administration of corresponding antagonists.

Prostaglandins of E.series are potent vasodilators and they cause a fall in blood pressure due to decreased peripheral resistance.[11] From among the antagonists of pro staglandins, Flufenamic acid (Fenamates) has been tried as an antagonist to various effects of Prostaglandins.

The hypotensive effect produced by garlic extract in dogs was not antagonized significantly by prior administration of flufenamic acid. Since polyphioretin phosphate is the only specific antagonist for majority of smooth muscle effects of pro staglandins, the possibility that garlic extract acts like prostaglandins cannot be ruled out.

Since the actions of garlic extract on ratuterus and guinea-pig ileum were blocked by flufenamic acid, it may be postulated that the garlic extract acts like prostaglandins to produce its hypotensive effect.

REFERENCES

1. Nadkarni, AX. Indian materia medica. 3rd ed. Bombay, population Book Depot, 1954, vol. 1.
2. Stoll, A. and seeback, E. Chemical investigations of AlIin, the specific principle of garlic. Advance EnzymoL, 1951; 11: 377.
3. Bordia, A., Bansal, H.C., Arora, S.K. and Singh, S.V. Effect of essential oils of garlic and onion on alimentary hyperlipemia. Atherosclerosis, 1975;21 : 15.
4. Malik, Z.A. and Siddiqui, S. Hypotensive effect of freezedried garlic (Allium sativum) sap in dog. JPMA., 1981;31 : 12.
5. Sial, A.Y. and Ahmad, S.l. Study of the hypotensive action of garlic extract on experimental animals. JPMA.,1982;32:237.
6. Sial, A.Y. Study of the hypotensive action of garlic extract on experimental animals. Karachi, JPMC-Department of Pharmacology, 1981.
7. Quffliam, J.P. Paraldehyde and methyl pentynol and ganglionic transmission. Brit. J. Pharmacol., 1959; 14 : 277.
8. Folle, L.E. and Aviado, D.M. The cardiopulmonary effects of quinnidine and procainamide. J,PharmacoL, 1966; 154 :92.
9. Pharmacological experiments on isolated preparations. By staff of department of Pharmacology and Therapeutics, University of Edinburg, 1976.
10. Ash, A.S.F. and Schild, H.O. Receptors mediating some actions of histamine. Br.J.Pharmacol., 1966;27 :427.
11. Karim, S.M.M. The prostaglandins, Progress in research. Medical and technical publishing, Oxford and Lancaster, 1972.