

# BIOASSAY FOR PROSTAGLANDIN-LIKE ACTIVITY OF GARLIC EXTRACT USING ISOLATED RAT FUNDUS STRIP AND RAT COLON PREPARATION

Pages with reference to book, From 138 To 141

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## Abstract

Hypotensive effect of garlic extract involving releasing of various prostaglandins is well documented. To further evaluate the prostaglandin-like activity of garlic extract, its effect has been demonstrated using isolated rat fundus strip and rat colon preparation. Garlic extract produced the effects on the isolated tissue similar to those of PGE. The use of this response as a bioassay for prostaglandin is discussed (JPMA 36:138: 1986).

## INTRODUCTION

Garlic (*Allium sativum*) is commonly used as a spice in our food. Apart from this use, it has been used as a folk medicine in India and China. Modern scientific research has proved many useful medicinal properties of garlic. It acts as an anti-septic and spasmolytic and is effective against bronchial Asthma<sup>1</sup>. Garlic has also stimulant, carminative, hypolipaeamic, antiatherosclerotic and antihypertensive properties<sup>2,3</sup>. Due to medicinal importance of garlic, extensive research is going on in Pakistan and abroad. Now a days hypotensive effect of garlic extract is being evaluated by various research workers. Our objective in this study was to evaluate some aspects of the mechanisms of hypotensive effect of garlic extract in detail.

## MATERIALS AND METHODS

**i) Garlic Extract:** Garlic extract was prepared by method as described by - Sial<sup>4</sup>. Peeled, uncrushed cloves of garlic were extracted thrice with absolute alcohol with an interval of 48 hours between each extraction. The combined alcoholic extract was freed of solvent and residue was partitioned between ethyl acetate and water. The soluble portion was freed of solvent and the residue was divided into alcohol soluble and alcohol insoluble portions. Water soluble but alcohol insoluble fraction has been 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, until used. Working dilutions were made with normal saline before use.

**ii) Rat Fundus Strip Preparation<sup>5</sup>:** The rat was kept fasting for 24 hours (except access to the water). The rat was killed by a blow on the head and cutting the throat. The abdomen was opened by an incision, the fundal part of stomach which is of grey colour can be easily distinguished from the pyloric part (pink colour). The fundal part was opened longitudinally, placed in a dish containing cold oxygenated Krebs's solution and made strips of 4 or 5 cm long by suitable transverse cuts. A thread was attached 'to one end of the tissue holder in the isolated organ bath and preparation was mounted in Krebs's solution (25 ml) at 37°C and aerated with oxygen nozzle while the other end was tied to the hook of myograph transducer. The myograph transducer was connected by a cable to the myograph coupler and the contractions of fundus strip were recorded on a polygraph. Myograph coupler was calibrated in such a way that 0.5g tension on thread holding the tissue was equal to 2 cm, of pan

deflection in upward direction. The preparation was equilibrated by 30 minutes rest before the administration of drug solution. The effect of garlic extract in different doses was observed in different rats. A time cycle of 10 minutes was observed in between two doses of garlic extract each time. Garlic extract was allowed to act for a period of 3 minutes.

**iii) Rat Colon Preparation<sup>5</sup>:** The rat was kept fasting for 24 hours (except free access to water). The animal was killed by a blow on the head and cutting the throat. The abdomen was opened, by an incision. The colon was identified, freed of mesocolon and a one inch long piece of colon was made. A thread was attached at each end and the preparation was mounted in Tyrode's solution (25 ml) at 37°C aerated with oxygen. One end of colon was tied to a hook of myograph transducer. The myograph transducer was connected by a cable to myograph coupler and the contractions of the colon were recorded on polygraph. Myograph coupler was calibrated in such a way that 0.5g tension on thread holding the tissue was equal to 2 cm deflection of writing pen in upward direction. The preparation was equilibrated by 30 minutes rest before administration of drug solution. A time cycle of 10 minutes was observed in between two doses of garlic extract each time. Garlic extract was allowed to act on tissue for a period of 3 minutes.

## RESULTS

i) Rat Fundus Strip: Garlic extract in doses of 0.2, 0.4, 0.8, 1.6 and 3.2 mg/25 ml produced contractions of rat fundus strip whose mean amplitudes in mm were  $3.00 \pm 0.40$ ,  $6.25 \pm 0.47$ ,  $10.75 \pm 0.62$  and  $15.25 \pm 0.40$  respectively (Table 1, Figure 1).

**TABLE-I**

### **Rat Fundus Strip Preparation : Effect of Garlic Extract.**

Dose of Garlic Extract mg/25 ml	No. of Experiments	Amplitude of Contraction in mm
0.2	4	$3.00 \pm 0.40^*$
0.4	4	$6.25 \pm 0.47^*(a)$
0.8	4	$10.75 \pm 0.62^*(a)$
1.6	4	$15.25 \pm 0.40^*(a)$
3.2	4	$15.25 \pm 0.40^*(a)$

\* represents Mean  $\pm$  SEM

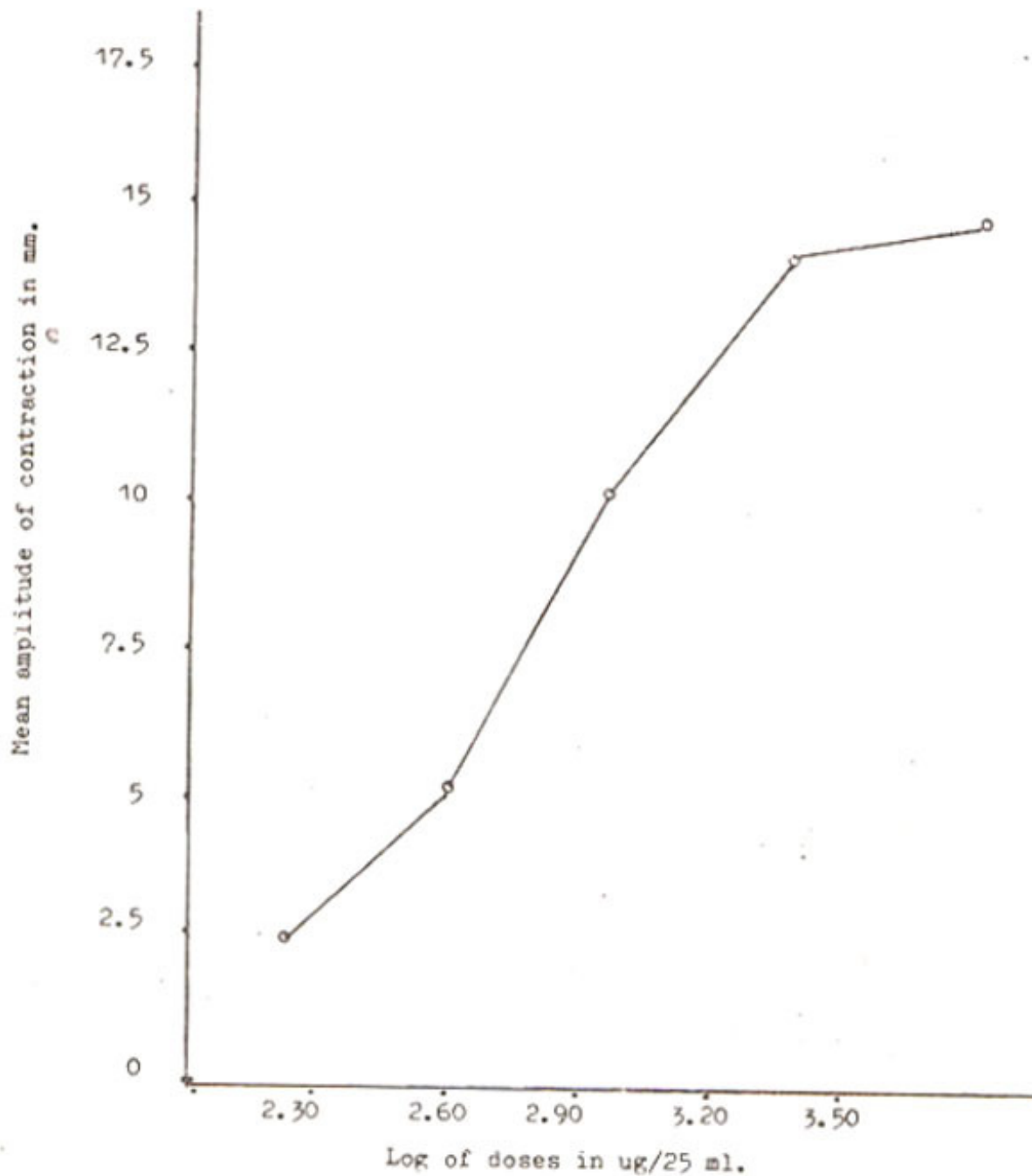


Figure 1. Log dose response curve showing the effect of garlic extract on rat fundus strip.

The amplitudes of contractions produced by these five doses of garlic extract on rat fundus strip were statistically highly significant ( $P < 0.001$ , Table 1).

ii) Rat Colon Garlic extract in the doses of 0.4, 0.8 and 1.6 mg/25 ml produced decrease in spontaneous colonic contractions, whose mean percent values were  $40.95 \pm 3.38$ ,  $79.87 \pm 2.27$  and 100 respectively (Figure 2, Table II),

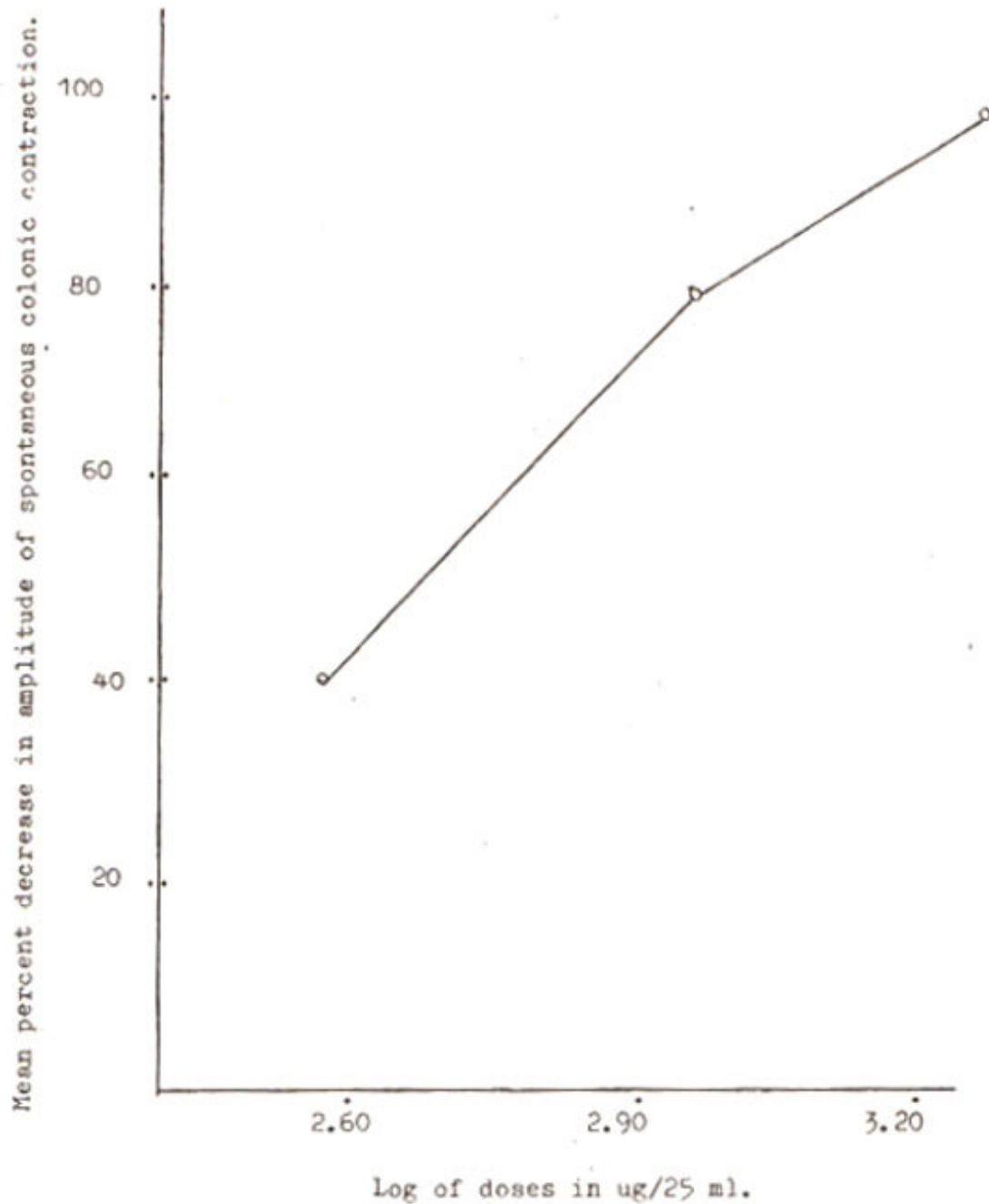


Figure 2. Log dose response curve showing the effect of garlic extract on rat colon.

**TABLE II**  
**Rat Colon Preparation: Effect of Garlic Extract.**

Dose of Garlic Extract mg/25 ml	No. of Experiments	Percent Decrease in Amplitude of Contraction
0.4	4	40.95 ± 3.28*(a)
0.8	4	79.87 ± 2.27*(b)
1.6**	4	100 (b)
3.2**	4	100(b)

\* Mean ± SEM

\*\* Those doses exerted complete inhibition.

(a)  $p < .01$

(b)  $p < .001$

The dose of 0.4 mg/25 ml produced mean percent decrease in the amplitude of spontaneous colonic contraction which was statistically significant ( $P < 0.01$ , Table II). The doses of 0.8 and 1.6 mg/25 ml produced mean percent decreases in the amplitudes of spontaneous colonic contraction, which were statistically highly significant ( $P < 0.001$ , Table II).

## DISCUSSION

Garlic extract caused contractions of fundus strip while relaxation of colon. This observation further strengthens the view that garlic extract has got prostaglandin activity. Prostaglandin E (PGE) causes contraction of fundus strip while relaxation of colon<sup>6</sup>. The chemistry of garlic<sup>7</sup> reveals presence of sulphur containing fatty acids and various actions of garlic extract were assigned to these fatty acids, (active principles). Prostaglandins are also fatty acids.

The idea that garlic extract behaves like prostaglandin can be confirmed by gas liquid chromatography, which at present, is not feasible because of non-availability of highly purified prostaglandins.

Prostaglandin-like activity can be detected and assayed by sensitive biological method<sup>6</sup> employing such isolated tissues as the ileum (rat, guinea pig), colon (rat), gastric fundus and uterus (rat).

Prostaglandins may be distinguished from other smooth muscle stimulants by using a suitable range of tissues and by selective use of blocking agents (e.g., atropine for acetylcholine, mepyramine for

histamine, methylethylamine for serotonin). Prostaglandins of different types may first be separated by differential extraction or by chromatography. Assays of PGE<sub>2</sub> and PGF<sub>2</sub> in mixture can be carried out by using two tissues, the rat fundus and colon. PGE contracts the rat fundus while relaxes the rat colon, where PGF<sub>2</sub> contracts the rat colon while relaxes the rat fundus.<sup>6</sup>

There is considerable evidence regarding the hypotensive effect of the garlic extract. Administration of the extract (I. V) produces hypotension in dogs. The mechanism of this hypotensive effect in dogs was earlier thought to be due to endogenous release of various hypotensive substances in the body<sup>8</sup>. Later it was postulated that hypotensive effect of the garlic extract may either be due to its prostaglandin-like content or by release of prostaglandins in the body<sup>9</sup>. The effect of garlic extract on isolated rat fundus strip and rat colon are just similar to those of PGE<sub>2</sub>. This finding also supports one previous postulation of prostaglandin-like activity in the garlic extract.

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