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Dose Response Curve of Acetylecholine, Its Modification by Water Extract of *Allium Sativum* and Estimation of PD2 Value Taking Atropine as A Standard Drugs

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ABSTRACT

This research aims to study how the water extract of *Allium sativum* (garlic) affects muscle contractions caused by acetylcholine (ACh) in isolated chicken intestines, using atropine as a comparison drug. The experiment included documenting dose-response curves (DRC) of acetylcholine both alone and in conjunction with atropine (a recognized competitive muscarinic antagonist) and *Allium sativum* aqueous extract (0.5 mL). The contractile responses were documented in arbitrary units, and PD_2 values were computed to evaluate antagonist potency. The DRC of ACh alone displayed a characteristic sigmoidal pattern with escalating muscular contraction, resulting in a PD_2 value of 5.075. The addition of atropine resulted in a rightward shift of the DRC, yielding a PD_2 value of 5.375, so confirming its potent competitive antagonism. Correspondingly, the DRC with *Allium sativum* extract exhibited a rightward shift, yielding a PD_2 value of 5.125, indicating considerable anticholinergic action. The findings suggest that *Allium sativum* contains active compounds that might reduce the effects of ACh on muscarinic receptors, but they are not as strong as atropine. The research supports the traditional use of garlic for easing stomach cramps and suggests it could be a natural source of substances that block muscarinic activity. Additional research is necessary to identify the precise phytoconstituents responsible for the reported effects and to examine their therapeutic significance. There was a very little evidences are found of anticholinergic activity towards the different concentration of Ach on chicken ileum and its modifications in response to water extract of *Allium sativum.* This research aims to study how the water extract of *Allium sativum* (garlic) affects muscle contractions caused by acetylcholine (ACh) on isolated chicken intestine. Here Atropine was applied as model standard drug to compare the ant cholinergic effect of garlic water extract.

Keywords: *Allium sativum (garlic), dose-response curves (DRC), acetylcholine, atropine, PD_2 value, Antagonist activity, Chicken ileum, anticholinergic*

1. INTRODUCTION

The autonomic nervous system plays a significant role in gastrointestinal motility, with the cholinergic pathway being a major modulator via muscarinic M_3type receptors [1]. Anticholinergic agents inhibit acetylcholine's action at these receptors, leading to relaxation of smooth muscles such as the ileum, which makes isolated ileum preparations a useful experimental model in pharmacological research [2] Garlic (*Allium sativum*) is a widely used medicinal plant known for it's therapeutic properties, particularly its antimicrobial, antioxidant, and cardiovascular benefits, primarily attributed to organosulfur compounds such as allicin, alliin, and ajoene [3,4]. A Dose-Response Curve (DRC) is a graph that plots the magnitude of a biological response (e.g., contraction of ileum) against increasing concentrations of a drug (usually plotted on a logarithmic scale). In pharmacology, DRC help to determine the potency (EC_{50}) and efficacy of a drug. As an agonist (e.g., acetylcholine),

shows a sigmoidal curve that rises as the dose increases. Modification of DRC occurs when another substance is introduced with Acetylcholine, in case of a competitive antagonist causes a rightward shift of the DRC without reducing the maximum response. A non-competitive antagonist causes a flattening or decrease in the maximum response [5].

PD_2 Value refers to the **negative logarithm of the molar dose of agonist** (usually ACh) producing 50% of the maximal response and sometimes used in agonist-response studies,[6]. In **water extraction, where plant material is soaked or boiled,heated at 80^{0}C in distilled water** as the solvent suitable for extracting **water-soluble compounds like** Alkaloid, flavonoids and saponins etc . **Decoction is** a traditional method especially useful for tough plant materials (e.g., bulbs, roots) involves **boiling** the plant material in water for 15–60 minutes, followed by **cooling, filtering,** and sometimes **concentration.[7] Chicken Ileum** has got **muscarinic receptors (M_3) which** responsive to acetylcholine. It's Produces **reliable, contractile responses** suitable for evaluating Anticholinergic activity, Spasmolytic effects. **Chicken** Ileum segment mounted in **organ bath and** Contractile response to ACh recorded using **kymograph.**. [8, 9]

2. MATERIALS AND METHOD

Fresh *Allium sativum* (garlic) bulbs were sourced from a local market from Kishanganj, Biharand were identified at the Department of Biological Sciences, University of Mata Gujri University, Kishanganj.

2.1Preparation of Allium sativum (Garlic) extract of infusion methods

The bulbs were cleaned under running distilled water to remove dirt and surface contaminants. Cloves were separated, peeled, and air-dried at room temperature (25 ± 2°C) to avoid loss of volatile bioactive components and preserve thermolabile compounds [10,11].For the infusion method, 50 grams of freshly crushed garlic cloves were weighed and transferred into a clean 1-liter beaker. To this, 500 mL of freshly boiled distilled water (approximately 80°C) was added. The mixture was immediately covered to minimize the loss of volatile constituents and allowed to stand for 24 hours at room temperature, away from direct sunlight [11]. Intermittent stirring was performed every 2–3 hours to enhance solvent penetration and extraction efficiency [12]. After the 24-hour extraction period, the infusion was first filtered through muslin cloth to remove large particulates, followed by fine filtration using filter paper. The clear aqueous extract was collected and stored in sterile, amber-colored glass containers at 4°C to prevent microbial growth and photodegradation of bioactive compounds [13].

2.2 Phytochemical Characterization of Garlic Extract

Phytochemical Test was performed on the garlic (*Allium sativum*) extract obtained via infusion to identify the presence of bioactive constituents. These tests were conducted using standard qualitative procedures as described in phytochemical Charaterzation [14, 15, 16, 17} Table 1 manuals

Table.1 Determination of Phytochemical group of infusion extract of Garlic (Allium sativum)

Photochemical	Test Performed	Result
Alkaloids	Wagner's test	Positive
Phenolics	Ferric chloride test	Positive
Flavonoids	Alkaline reagent test	Positive
Saponins	Froth formation test	Positive

+ = Positive, - = Not detected /absent

Isolated chicken ileum preparation

The Fresh ileum (part of small intestine) was collected from a chicken bought at the local market (Kishanganj, Bihar) & kept in Tyrode solution (NaCl, 136.7; KCl, 2.68; MgCl2.2H2O, 1.05; NaH2PO4, 0.42; CaCl2.2H2O, 1.80; NaHCO3, 11.90; Glucose, 5.55Mm) and cleaned off the mesentery[18,19]. The segment of 2 cm long was mounted in a 30 ml tissue organ bath and maintained at 37 ℃. . Dose response curve of acetylcholine was recorded on the kymograph first. Then cumulative concentration-effect curves were recorded on kymograph for Acetylcholine in absence and presence of infusion extracts of

Garlic (*Allium sativum*) on Kymograph by using Sherrington's Recording Drum [20]. The same procedure was carried for concentration effect curve of Ach in presence of Atropine sulphate as a standard drug. The percentage inhibition of extract and standard drug was calculated and graph was plotted by taking log dose versus height of response curve [21, 5].

Experimental condition: The kymograph is smoked by gradually rotating the drum while exposing it to a paraffin or kerosene flame to create a consistent coating of soot. This enables recording levers to document muscle contractions directly onto the smoked surface during physiological tests. Post-recording, the trace is preserved using shellac. This technique is essential for viewing and analysing smooth muscle responses in traditional pharmaceutical contexts [23, 24].DRC was taken after proper base line. Rotation speed of the drum was 0.25mm/sec. Magnification was kept as 10 and 90 second response cycle with 3 successive washing.

Table 2: Dose response relationship observations of Ach, Water extract of Garlic *Allium sativum* and atropine on chicken ileum.

Conc of Ach µg/ml	in ml	Resp-onse in mm	Atro-Pine 0.5 ml	Ach + Extract 0.5ml	Conc. of Ach µg	Conc. of Ach µg/ml in organ bath	Conc. of Ach (µM/L)	Conc. of Ach (µM/ml)	Log Conc.	% Resp-onse	% Response Ach + Atp 0.5 ml	% response Ach + Extract
50	0.05	**5**	**4**	**5**	**2.5**	**0.083**	**0.00046**	**4.6000E-07**	**-6.3372**	**7.35**	**5.88**	**7.353**
50	0.1	**7**	**5**	**6**	**5**	**0.167**	**0.00092**	**9.20E-07**	**-6.0362**	**10.29**	**7.35**	**8.824**
50	0.2	**10**	**7**	**9**	**10**	**0.333**	**0.00184**	**1.84E-06**	**-5.7352**	**14.71**	**10.29**	**13.235**
50	0.4	**17**	**12**	**14**	**20**	**0.667**	**0.00368**	**3.68E-06**	**-5.4342**	**25.00**	**17.65**	**20.588**
50	0.8	**28**	**16**	**25**	**40**	**1.333**	**0.00736**	**7.36E-06**	**-5.1331**	**41.18**	**23.53**	**36.765**
50	1.6	**58**	**25**	**38**	**80**	**2.667**	**0.01472**	**1.47E-05**	**-4.8321**	**85.29**	**36.76**	**55.882**
100	1.6	**65**	**28**	**49**	**160**	**5.333**	**0.02944**	**2.94E-05**	**-4.5311**	**95.59**	**41.18**	**72.059**
200	1.6	**68**	**29**	**53**	**320**	**10.667**	**0.05888**	**5.89E-05**	**-4.2300**	**100.00**	**42.65**	**77.941**
400	1.6	**68**	**29**	**53**	**640**	**21.333**	**0.11776**	**1.18E-04**	**-3.929**	**100.00**	**42.65**	**77.941**

									0			

DRC of Acetylcholine:

The isolated chicken ileum showed a typical sigmoidal contractile response to increasing concentrations of acetylcholine (ACh), as expected due to activation of muscarinic M_3 receptors in smooth muscle. ACh-only responses (arbitrary units) are 5, 7, 10, 17, 28, 58, 65, 68, and 68. The response increased progressively with dose, indicating receptor activation and saturation at higher doses. The curve plateaued at doses 8th (10.667µg/ml) and 9th (21.333 µg/ml) dose of, suggesting a maximal cealing effect had been reached. The PD_2 value of ACh alone was calculated to be 5.075, indicating the log of the inverse molar concentration of ACh required to produce 50% of the maximal response.

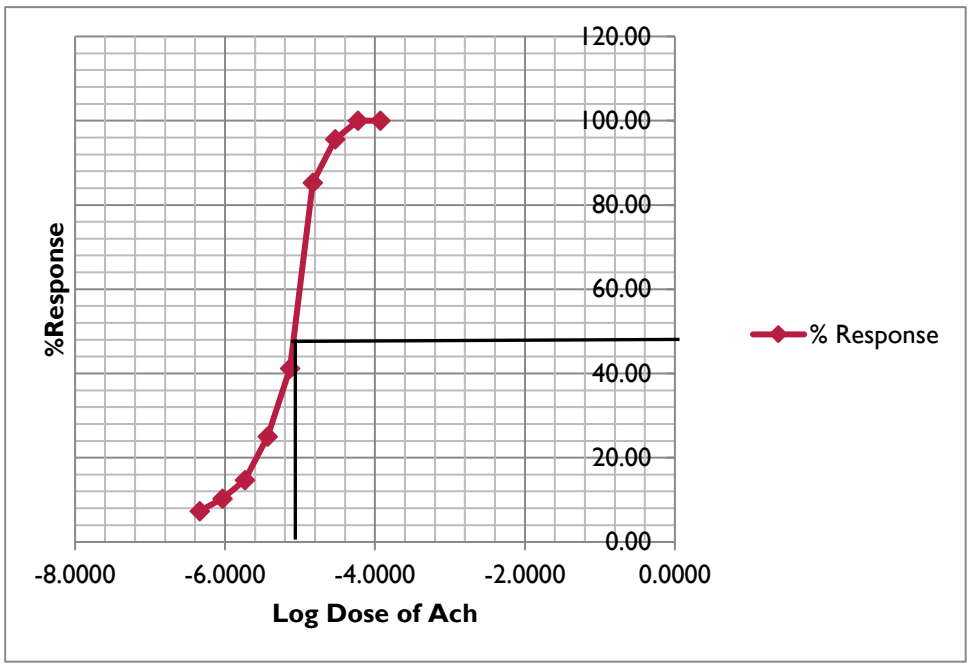


Fig 1: Log Dose of Ach

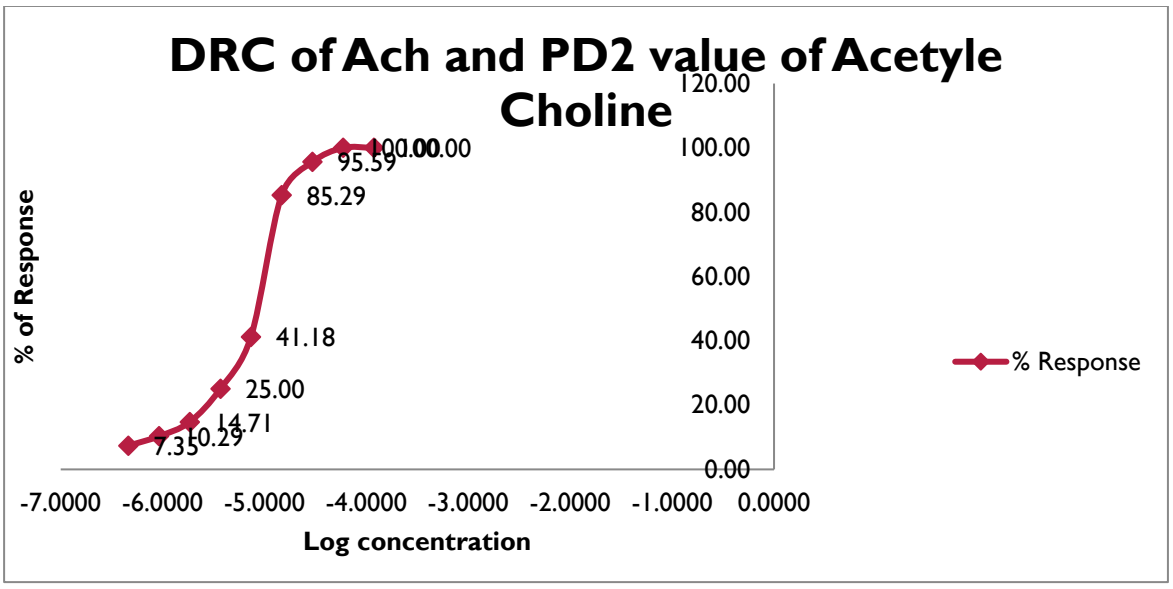


Fig 2: DRC of Ach and PD2 value of Acetyle Choline

Modification of DRC Ach and its modifications by test and standard drug:

Response of same dose of Acetylecholine were recorded in presence of 0.5 ml Atropine standard stock solution, ACh + Atropine responses were 4mm , 5mm, 7mm, 12mm, 16mm, 25mm, 28mm, 29mm and 29mm. There was **a clear rightward shift** in the DRC compared to ACh alone, with a similar maximal sharp reduction in maximal effect. This demonstrates **competitive antagonism,** as atropine binds to muscarinic receptors without reducing the efficacy of ACh. The **PD_2 value increased to 5.375**, confirming atropine's role as a **potent competitive antagonist** that increases the amount of ACh required to produce the same effect.

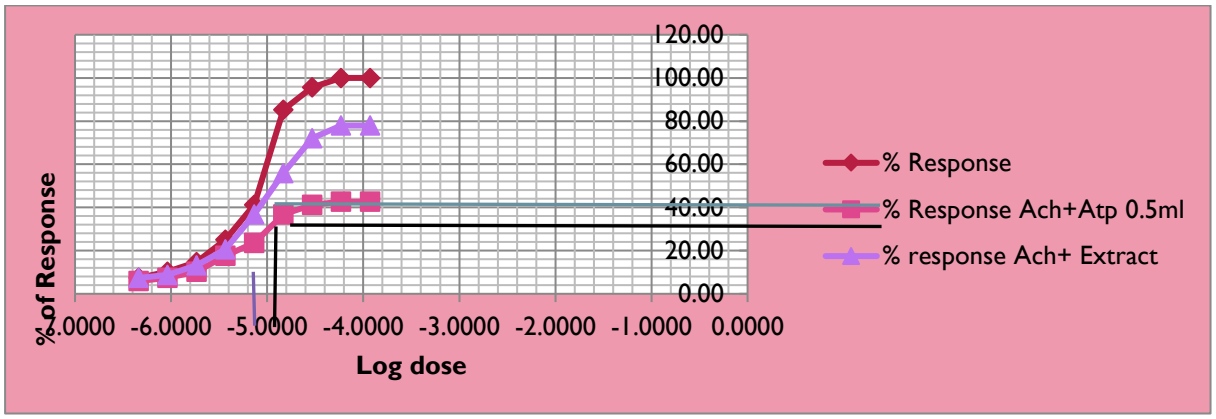


Fig 3: Log Dose of Ach, Modification by Atropine and *Allium sativum* water extract 0.5 ml

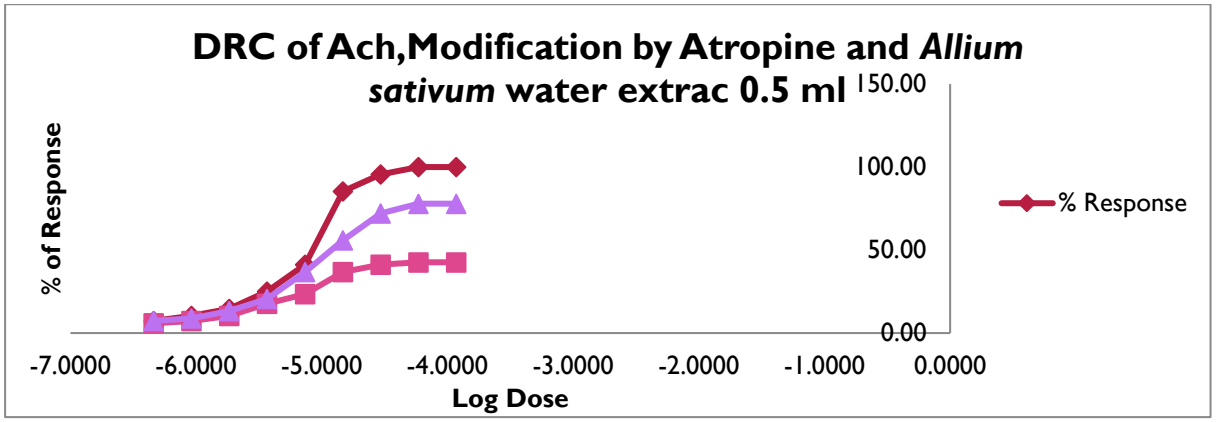


Fig 4: Comparative dose response relationship of Ach, Water extract of Garlic *Allium sativum* , and atropine on chicken ileum

PD2 value of Ach

5.075

PD2 value Ach in presence if Atp

5.375

pD2 value Ach in presence if *Allium sativum* water extract 0.5ml

5.125

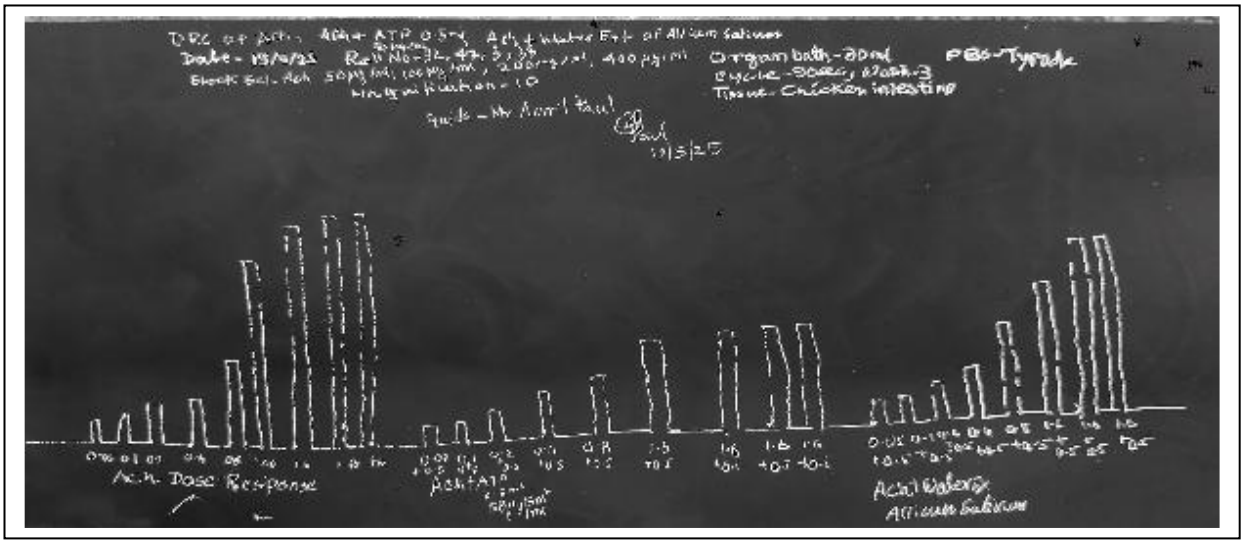
Formulation of DRc Ach & Garlic extract (Allium sativum):

Same above mentioned process was again repeated with garlic water extracts 0.5 ml with same dose of Ach. 5mm, 6mm, 9mm, 14mm, 25mm, 38mm, 49mm, 53mm and 53 mm consecutive response an a moderate rightward shift of DRC was noted. The sharp fall of with a slight reduction in maximal response compared to ACh alone suggests the garlic extract may

act as a partial competitive antagonist, possibly via muscarinic receptor blockade or may be other mechanism. The PD_2 value was found to be 5.125, indicating moderate anticholinergic activity, less potent than atropine but still significant.

3. DISCUSSION AND CONCLUSION

This study looked at how the water extract of *Allium sativum* (garlic) containing Alkaloid, flavonoids,saponines and phenolic compound affects the anticholinergic properties on chick illum acetylcholine (ACh) with atropine, which is a known muscarinic antagonist. The study looked at how the dose-response curve (DRC) and the related PD_2 values changed to measure how strong the antagonist was. When acetylcholine was administered alone. The PD_2 score was 5.07, indicating enhanced smooth muscle contraction with escalating dosages. ACh effectively induces 50% of the maximum contraction. Atropine was delivered with acetylcholine. The PD2 score rose to 5.375, indicating competitive antagonism. The high affinity of atropine for muscarinic receptors causes a rightward shift in the dose-response curve. We administered the *Allium sativum* water extract solution along with acetylcholine. The PD2 score was 5.125, influencing smooth muscle contraction and showing moderate antagonistic activity. The *Allium sativum* extract solution partly inhibits muscarinic receptors or affects downstream mechanisms. The increase in PD_2 value after adding *Allium sativum* extract shows that ACh is less effective, confirming that the extract has anticholinergic properties. Even though it wasn't as strong as atropine, the garlic extract showed a significant change in how the body responds to cholinergic signals, possibly affecting muscarinic receptors or how smooth contract muscles.



4. CONCLUSION

The study concludes that the water extract of *Allium sativum* exhibits **moderate anticholinergic activity,** as shown by the rightward shift of the ACh dose-response curve and an increased PD_2 value. While not as potent as atropine, garlic extract shows potential for use in conditions involving smooth muscle spasms, supporting its **traditional medicinal applications.** Further experimental works may give a clear view about the dose response relationship and category of antagonism of the *Allium sativum* water extract possessed

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