

Full Length Research Paper

Hypotensive effect of an aqueous extract of *Heliotropium indicum* Linn (Boraginaceae)

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Accepted 02 April, 2012

Heliotropium indicum Linn (Boraginaceae) has been used widely in the treatment of hypertension in Ghana. This study therefore was aimed at investigating the effects of an aqueous extract of *H. indicum* Linn on the heart and blood pressure of some laboratory animals as a means of establishing its hypotensive effect and verifying its traditional medicinal use as an antihypertensive. The extract was applied *in vitro* to an isolated frog and rabbit heart to investigate its effect on the force and rate of cardiac contraction, and administered to an anaesthetized cat to establish its effect on blood pressure. The extract had a dose-dependent negative inotropic and chronotropic effect on the frog and rabbit hearts similar to that observed for acetylcholine and histamine. These effects were inhibited by atropine but not mepyramine suggesting a muscarinic-receptor mediated effect. *In vivo*, the *H. indicum* aqueous extract produced a dose-related reduction in blood pressure of the anaesthetized cat. Findings reveal that *H. indicum* has hypotensive effect suggesting its usefulness in the treatment/management of hypertension and other cardiovascular diseases.

Keywords: Cardiovascular disease, Chronotropic effect, Hypotensive, Inotropic effect, Heart, Acetylcholine, Histamine, Muscarinic activity

INTRODUCTION

Hypertension is a highly prevalent cardiovascular disease risk factor in developing countries and a primary risk factor for heart disease and stroke, the leading causes of death worldwide (Chockalingam *et al.*, 2006). The World Health Report identified hypertension as the third ranked factor for disability-adjusted life years (Chockalingam *et al.*, 2006). Recent analyses have shown that as of the year 2000, there were 972 million people living with hypertension worldwide, and it is estimated that this number will escalate to more than 1.56 billion by the year 2025

(Kearney *et al.*, 2005; Koffuor *et al.*, 2011).

Hypertension is now widely reported in Africa and is the most common cause of cardiovascular disease on the continent. It is estimated that more than 20 million Africans are affected, majority of which live mainly in urban areas (Addo *et al.*, 2007). Prevalence ranges from 25-35 % in adults aged 25-64 years, and its prevention and control could avoid at least 250,000 deaths in Africa per year (Koffuor *et al.*, 2011).

In Ghana, the prevalence of hypertension in urban Accra is 28.3 % (crude) and 27.3 % (age-standardized) (Amoah, 2003). In Kumasi and surrounding communities, the prevalence is 28.7 % (Cappuccio, 1997). The Ghana Health Service indicates that hypertension is the number one killer disease in Ghana

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today. It is now the second most reported medical condition but was the fifth as at 2006 (GHS, 2007). The upsurge of hypertension has led to an increase in the demand for alternative antihypertensive medicines.

Heliotropium indicum Linn (Family Boraginaceae) commonly known as the “Cock’s comb” and called locally in Ghana as “*Akonfem atiko*” (Akan-Asante) (Burkill, 1985). This plant is widely used in West Africa, India and the Philippines. Its leaves have traditionally been used in medicine as abortifacients, ecboolics, antidotes (venomous stings, bites), anti-arthritics, anti-rheumatics, eye treatments, fabrifuges, analgesics, and vermifuges. It has been used in managing epilepsy and convulsion. The whole plant has activity against diarrhea, dysentery, tumours and cancers (Burkill, 1935; Ainsle, 1937; Dalziel, 1937; Quisumbing *et al.*, 1957; Oliver, 1960; Irvine, 1961; Kugelman, 1976).

The plant is used extensively among rural dwellers and some urban dwellers in Ghana, in treating hypertension. This study is therefore an attempt at establishing scientific backing for the use of extracts of *Heliotropium indicum* as an antihypertensive by investigating its effect on the activity of the frog and rabbit hearts, and its effect on blood pressure in the anaesthetized cat.

MATERIALS AND METHODS

Plant Collection

The aerial parts of *Heliotropium indicum* were collected from Buokrom, a suburb of Kumasi (the capital of the Ashanti Region), Ghana, in September, 2007 and authenticated by the Curator (Mr. Adator K. Brown) of the Department of Theoretical and Applied Biology, College of Sciences, KNUST, where a voucher specimen (KNUST/ BSC / F621) has been deposited.

Preparation of Aqueous Extract

Sun-dried aerial parts of *Heliotropium indicum* were comminuted to coarse powder by a hammer mill (Schutte Buffalo, New York, USA). A 600 g quantity of the coarse powder was also mixed with 5 liters of water and warmed for 15 minutes. The infusion was filtered to obtain a dark - brown filtrate, concentrated by evaporation over a hot water bath (B-Tex Laboratory Engineering, Gujarat India) and later in a Gallenkamp hot air oven (Oven 300 plus series, England) at 60 °C until a constant weight was obtained. It was cooled in a desiccator to yield dark - brown solid extract which was named HIE and will be referred to in this study as such.

Animals and Husbandry

Male frogs (*Rana ridibunda*) weighing 100–120 g, adult New Zealand white rabbits weighing 1.8-2.5 kg of either sex and cats of either sex weighing 3-4 kg were used for the experiment. The animals were kept in the Department of Pharmacology animal house under ambient temperature of 25-30 °C and relative humidity of 65-87 % with a normal 12-hour dark and light cycle and had free access to greens and water *ad libitum*. All procedures and techniques used in these studies were in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH, 1996). The protocols for the study were approved by the Departmental Ethics Committee.

Drugs and Chemicals

Acetylcholine, histamine, adrenaline, atropine, mepyramine heparin, and chloralose were obtained from Sigma-Aldrich Inc., St. Louis, MO, USA. Acetylcholine and histamine were used as reference drugs that cause negative inotropic and chronotropic effects and decrease blood pressure. Atropine and mepyramine are specific antagonists to these drugs. Adrenaline was used as reference drug that causes positive inotropic and chronotropic effects. Heparin was used as an anticoagulant and chloralose, an anaesthetic agent.

Preliminary Phytochemical Screening

HIE was screened for the presence of phytochemicals as described by Sofowora (1993), Harbone (1998), and Trease and Evans (1989).

Isolated Frog Heart Experiment

The pithed frog was placed on a frog board set up at room temperature (27-28 °C) and its chest carefully opened. The heart carefully freed from connective tissue (whilst ensuring that all blood vessels remain intact) and was exposed well enough for drug administration. The heart was continuously kept moistened by irrigation with frog - Ringer solution. A small platinum clip used to hold the base of the ventricles was tied with cotton thread to a lever system fitted with a frontal writing point moving on a Harvard kymograph (Harvard Apparatus Ltd, Kent, UK). The normal rhythmical force and rate of contractions (baseline) was recorded on a white paper wound

around the 30 cm diameter kymograph drum revolving at a speed of 4 mm per minute. Acetylcholine, histamine, adrenaline (1×10^{-3} – 3.2×10^{-2} mg/ml) and HIE (0.1 - 12.8 mg/ml) were each administered directly onto the frog heart preparation and the resultant rhythmical force and rate of contractions were recorded. Observing that the effects recorded for HIE was similar to that of acetylcholine and histamine, the effects of these three drugs were recorded again in the presence of atropine (8×10^{-6} mg/ml) and then mepyramine (1×10^{-7} mg/ml). The effect of adrenaline was recorded again in the presence of HIE (3.2 and 6.4 mg/ml) to check for a possible antagonism. Each procedure described above was repeated four times.

Perfused Rabbit Heart Experiment

The rabbit was injected with heparin (1000 IU) through the marginal ear vein (as a precaution to prevent clot formation in the heart) after which it was exsanguinated. The heart with at least 1 cm of aorta attached removed as quickly as possible and placed in a dish of Ringer Locke physiological solution maintained at 37°C. The aorta was located and dissected free of all connective tissues. All other vessels connected to the heart were trimmed away. The aorta was cut just below the point where it divides and the heart transferred to the perfusion apparatus where the aorta was tied firmly on a glass cannula with a thread. Care was taken to ensure that no air bubble got into the aorta however bubbles formed in the glass cannula were removed by a thin long polythene pipette. The perfusion fluid from a reservoir was oxygenated at a constant pressure and maintained at 37 °C. A clip was attached to the tip of the ventricle of the heart and connected to a spring lever by a thread and the amplitude of contractions of the heart were recorded via a universal Oscillograph (model 50 - 8622, Harvard Apparatus Limited, Kent, England). The rate of flow was measured by placing a graduated cylinder beneath the lower chamber of the preparation. This was possible because the pressure of the perfusion fluid closed the aortic valve, forcing fluid to pass through the coronary vessels, escaping from the inferior vena cava to form drops which dripped off into the graduated cylinder.

The contractions and perfusion rate were again recorded after applying acetylcholine (1×10^{-6} – 1×10^{-1} mg/ml) and HIE (0.1-100 mg/ml) to the rabbit heart preparation through the perfusion fluid. These effects were recorded again in the presence of atropine (8×10^{-6} mg/ml). Each procedure described above was repeated four times.

The Anaesthetized Cat Experiment

The cat was anaesthetized using 1 % w/v chloralose (80 mg/kg, i.p). The femoral vein on one of the hind legs was isolated by dissection and fitted with a cannula. The arterial blood pressure was recorded through a carotid artery cannula (polythene tubing, 1.02 - 1.27 mm) via a pressure transducer (model P23Gb, Statham Instruments, Inc.) and the phasic arterial pressure was continuously monitored via a universal Oscillograph (model 50 - 822, Harvard Apparatus Ltd, Kent, England).

Effects of doses of acetylcholine (1×10^{-8} - 1×10^{-1} mg/kg) and HIE (1 - 1000 mg/kg) on the arterial blood pressure was recorded. These effects were again recorded in the presence of atropine (1.2×10^{-6} mg/ml). It should be noted that after each drug administration, cardiovascular variables were allowed to stabilize (in about 15 minutes) before any further administration of drugs were made. The venous catheter was continuously flushed with 0.5 ml of 0.9 % normal saline after each dose administered, this was to ensure that drugs collected in the catheter were all pushed into the femoral vein. Each procedure described above was repeated four times.

The doses of agonists and antagonists were chosen based on preliminary studies in our lab.

Data Analysis

GraphPad Prism Version 5.0 for Windows (GraphPad Software, San Diego, CA, USA) was used for all data analyses and plotting of the graphs. Data are presented as mean \pm SEM.

RESULTS

Phytochemical Screening

Phytochemical screening of HIE reveal the presence of alkaloids, cyanogenic glycosides, tannins, saponins, and steroids.

The Frog Heart

Acetylcholine, histamine and HIE produced dose-dependent negative inotropic and chronotropic effects on the isolated frog heart (Figures 1 and 2). These effects were opposite to that produced by adrenaline. Antagonistic effects of atropine and mepyramine on

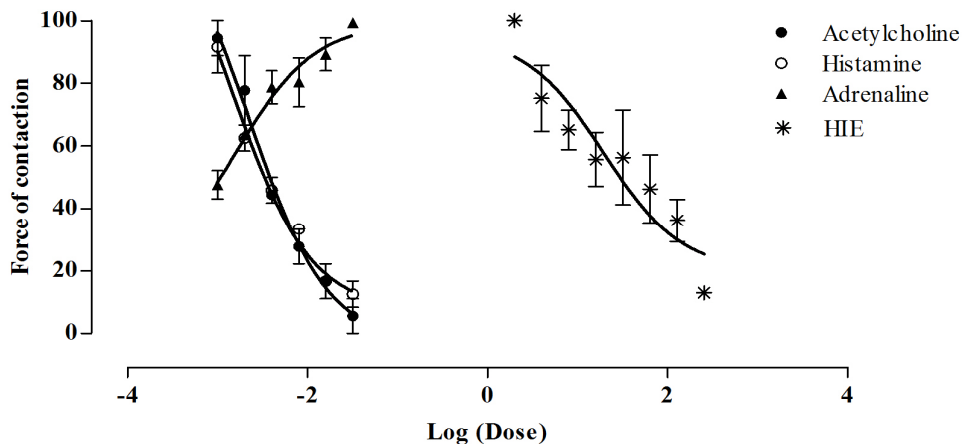


Figure 1. Log dose - response curves of acetylcholine, histamine, adrenaline (1×10^{-3} – 3.2×10^{-2} mg/ml) and HIE (0.1 - 12.8 mg/ml) on the force of contraction of the isolated frog heart. Each point is the mean \pm SEM (n = 4).

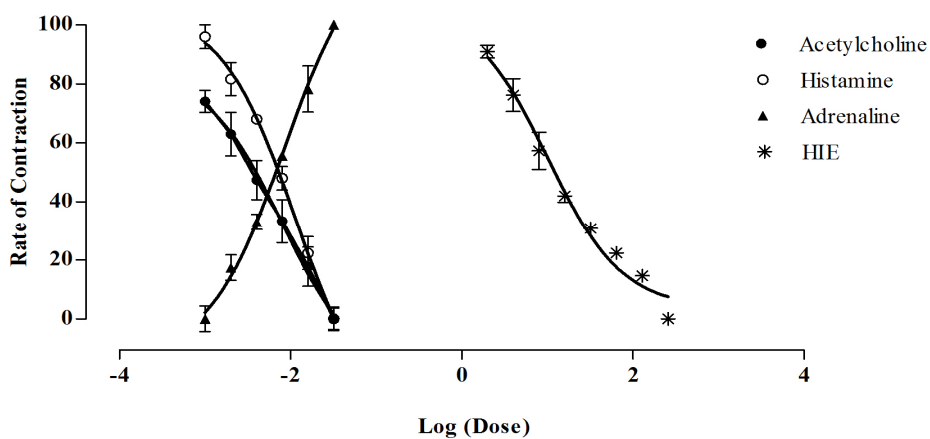


Figure 2. Log dose - response curves of acetylcholine, histamine, adrenaline (1×10^{-3} – 3.2×10^{-2} mg/ml) and HIE (0.1 - 12.8 mg/ml) on the rate of contraction of the isolated frog heart. Each point is the mean \pm SEM (n = 4).

Table 1. Effects of atropine and mepyramine on acetylcholine, histamine and HIE on the isolated frog heart

Antagonist Drug	Percentage Inhibition by Antagonist Drug		
	Acetylcholine	Histamine	HIE
Atropine	64.75 \pm 2.49	0	45.5 \pm 3.47
Mepyramine		55.75 \pm 3.47	

Values are means \pm SEM (n = 4).

their respective agonist drugs and on HIE is shown in Table 1 in which atropine inhibited significantly the effects of acetylcholine (64.75 ± 2.49) and HIE (45.5 ± 3.47). HIE had no inhibitory effect on the positive inotropic and chronotropic effects elicited by adrenaline.

The Perfused Rabbit Heart

HIE dose - dependently reduced the force of contraction and flow rate of the perfused rabbit heart similar to that of acetylcholine (Figures 3 and 4).

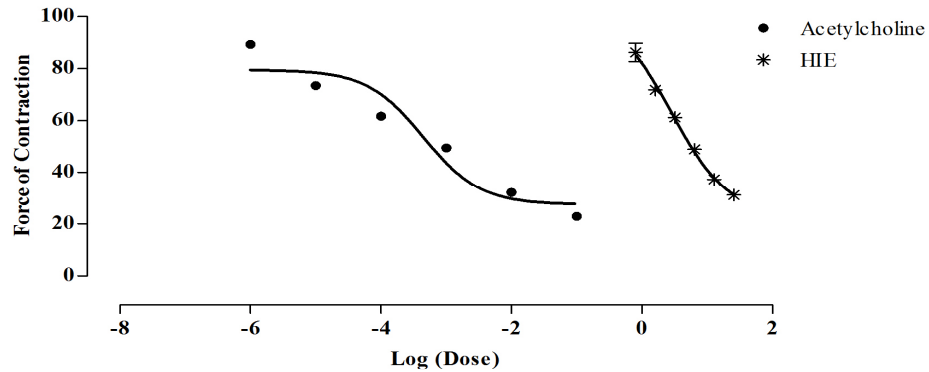


Figure 3. The effect of acetylcholine ($1 \times 10^{-6} - 1 \times 10^{-1}$ mg/ml) and HIE (0.1-100 mg/ml) on the contractions of the perfused rabbit heart

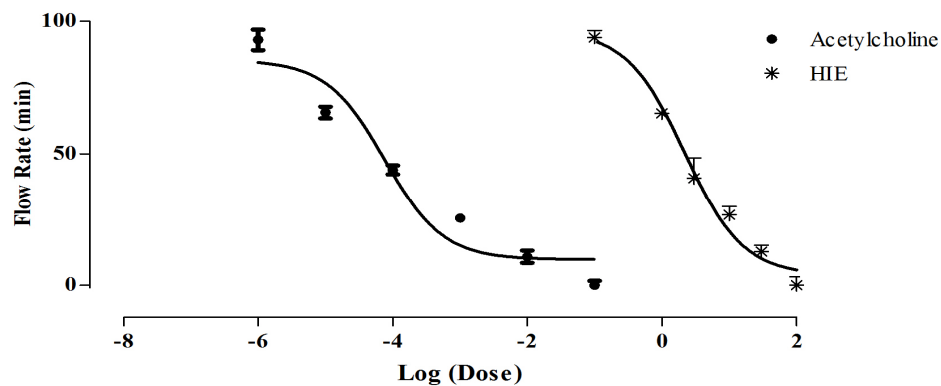


Figure 4. Effect of acetylcholine ($1 \times 10^{-6} - 1 \times 10^{-1}$ mg/ml) and HIE (0.1-100 mg/ml) on the flow rate of the perfused rabbit heart

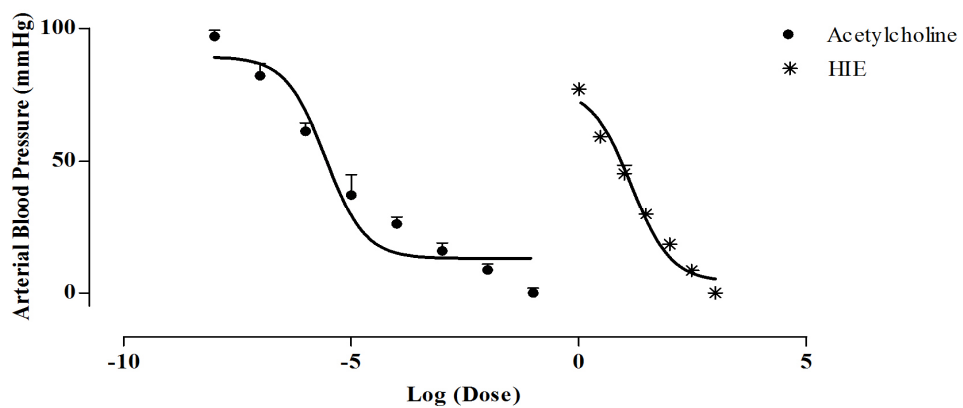


Figure 5. The effect of acetylcholine ($1 \times 10^{-8} - 1 \times 10^{-1}$ mg/kg) and HIE (1-1000 mg/kg) on the arterial blood pressure of an anaesthetized cat

The Anaesthetized Cat

HIE produced dose - dependent reduction in the arterial blood pressure of the anaesthetized cat comparable to acetylcholine (Figure 5). The depressor effects of

acetylcholine and HIE on the blood pressure of the anaesthetized cat were inhibited by $57.75 \pm 4.55\%$ and $48.25 \pm 3.56\%$ respectively, suggestive of muscarinic mediation.

DISCUSSION

This study has demonstrated that HIE has hypotensive properties due to its effect on the heart confirming the traditional use of the plant. The negative inotropic and chronotropic effect of HIE (similar to acetylcholine and histamine) on the heart could have contributed immensely to its hypotensive activity as blood pressure is the product of cardiac output and total peripheral resistance (Li *et al.*, 1998). By the negative effect HIE has on the heart, cardiac output will be reduced. A negative inotropic and chronotropic effect could be muscarinic- or histaminic-receptors mediated (Hartzell, 1980). The inhibition of HIE effects on the heart by atropine but not mepyramine suggests it muscarinic-receptor mediated effect.

Inhibition of the β -adrenoceptor activity on the heart can also lead to bradycardia (Klabunde, 2010; Sharma and Tarabar, 2011). In order to rule out the possibility of adrenoceptor inhibition by HIE, pre-administration of HIE was performed followed by adrenaline administration but HIE had no antagonistic effect on adrenaline activity which was a positive inotropic and chronotropic effect.

In the anaesthetized cat, mepyramine antagonized the pressure lowering (hypotensive) effect of histamine but not HIE whereas atropine blocked the hypotensive effects of both acetylcholine and HIE equi-effectively. This confirms the participation of the muscarinic receptors but not the histaminic and adrenergic receptors in the hypotensive effects of HIE. Tracing obtained on the universal oscillograph revealed again a negative effect on the heart.

Although the effect of the drugs on the total peripheral resistance (blood vessels) could not be measured in this study, the possibility of HIE having vasorelaxant effect on peripheral blood vessels cannot entirely be excluded from the observation that atropine reversed the effect of HIE in the anaesthetized cat and the vasorelaxant effect of acetylcholine is reported to be inhibited by atropine (Haghighi *et al.*, 2002).

The presence of some phytochemicals such as alkaloids, tannins, and saponins present in the extract may possibly be the sources of hypotensive effect. Some alkaloids are known to have hypotensive effect (Wu *et al.*, 1977) as some alkaloids are even known to have muscarinic activity (Wynter-Adams *et al.*, 1999). Some tannins have also been reported to have antihypertensive effect working via several mechanisms of action (Lin *et al.*, 1993; Liu *et al.*, 2003). Saponins have also been documented to have some hypotensive activity (Marston, 2005; Tiamjan *et al.*, 2007; Hiwatashi *et al.*, 2010).

CONCLUSION

The aqueous extract of *Heliotropium indicum* Linn produces a muscarinic-receptors mediated negative inotropic and chronotropic effects on the heart and has hypotensive effect *in vivo*. This extract could therefore be useful in treating/managing hypertension and possibly other cardiovascular disorders.

ACKNOWLEDGEMENTS

The Authors wish to express their gratitude to Mr. Thomas Ansa of the Department of Pharmacology, KNUST, Kumasi, Ghana and Mr. Adator K. Brown of the Department of Theoretical and Applied Biology, College of Sciences, KNUST, Kumasi, Ghana, for their technical assistance.

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