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Blood Pressure Lowering Effect of Extract of *Gongronema Latifolium.*

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ABSTRACT

The ethanol extract of *Gongronema latifolium* leaves were investigated for anti-hypertensive activity. To determine this, first ethanol extraction was carried out and the ethanol extract was further fractionated using three different solvents. N-hexane, chloroform and ethylacetate respectively and the residual washed with ethanol. The ability of different fractions to lower blood pressure of anaesthetized cat was carried out. The result obtained revealed that the crude ethanol extract and its four fractions significantly lowered the blood pressure of the cat. The extract and fractions effected blood pressure reduction in same way as the parasympathomimetic drug which activity was antagonized by atropine, indicating that the receptors were operating with the muscarinic receptors. The fractions of the crude in their own characteristic property lowered the blood pressure of anaesthetized cat. Of the four fractions, the chloroform fraction effected the highest blood pressure reduction followed by the n-hexane and then the ethylacetate fractions. The least reduction in blood pressure was by the residual ethanol fraction.

Keywords: *Gongronema latifolium*, Hypertensive activity, muscarinic receptors and parasympathomimetic.

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INTRODUCTION

The world has witnessed the emergence of non-communicable diseases which are the diseases "that appear at middle age after long exposure to an unhealthy lifestyle involving tobacco use, lack of regular exercise and consumption of diets rich in saturated fats, sugars and salts typified by fast foods" (Steyn and Damascena, 2006).

In the World Health report (2002), five of the ten leading global disease burden risk factors associated with non-communicable disease (NCD) are high blood pressure, high cholesterol, obesity, physical inactivity and insufficient consumption of fruits and vegetables. Hypertension (high blood pressure) is a disturbance in hemodynamic function in which there is a persistant abnormal elevation of systemic blood pressure (Aguwa, 1996) to a value exceeding 140/90 at rest (BMA, 2002). This progressive disorder, if not effectively treated results in increased probability of coronary thrombosis, stroke and renal failure (Rang et al., 1995) and high risk factor for cardiovascular morbidity and mortality (Horl, 2010).

It is now a leading cause of death in middle income countries and of emerging importance in low income countries (WHO, 2002, Rodgers et al, 2002). Conventional therapy for hypertension involving drugs therapy in lipid metabolism and cardiac regulation are froth with complications mostly of drug intolerance, drug reaction and toxicity (Shlafer and Marrieb 1989).

The role of diet in the aetiology of hypertension cannot be overemphasized. Where traditional diets have been replaced with high fat, high salt, high refined sugar diets and less fiber intake, there is prevalence of hypertension (Hu and Willet, 2002). Epidemiological studies have shown that there was a reduced rate of mortality due to coronary heart disease in Japanese population consuming a traditional diet compared with a Western diet (Kagan *et al*., 1974). Fruits and vegetables are important component of a healthy diet and their daily consumption could help to prevent major chronic diseases such as cardiovascular diseases (hypertension) (Ene Obong, 2001). Diets rich in fruits and vegetables as of Vegetarians (Sacks *et al.*, 1974), potassium (krishner and Kapor, 1991), fiber (Liu and Manson, 2001; He and Welthon, 1999) and phytochemicals such as flavonoids and carotenoids. Vitamins (Kirk *et al*., 1998; Agrawal and Rao, 2000) are associated with decreased total and low density Lipoprotein levels and prevention of atherosclerosis with subsequent reduction in arterial blood pressure.

Africa is endowed with many plants possessing medicinal properties and Nigeria is one such country, blessed with many plants that have long been recognized for their medicinal value (Iwu, 1993). One such vegetable is *Gongronema latifolium* a perennial, tropical plant with soft tissue listed among the African leafy vegetable (Smith and Eyzaguirres) and of medicinal importance (Ayodele, 2007). Its leaves and stem have been associated with anti-diabetic property (Ugochukwu and Babbady, 2003 and Ezewe, 2012), gastrointestinal relaxant (Gamaniel and Akah, 1996) and antimicrobial activity (Eleyinmi *et al.,* 2007).

It is with this knowledge that this work was undertaken to evaluate the possible blood pressure lowering effect of *Gongronema latifolium* on cats.

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METHODOLOGY

Material

Fresh leaf samples of *Gongronema latifolium Benth Asclepiadaceae* were purchased from vegetable market in Nsukka, Enugu state Nigeria. They were air-dried, pulverized and stored in a refrigerator.

Animals

Adult cats weighing between (2-2.3) kg were purchased from Ibagwa market in Nsukka area and housed in the Department of Pharmacology laboratory, University of Nigeria, Nsukka. The experiment animals were fed with food and water *ad libitum*.

Reagents

Acetylcholine (1mg/ml), Atropine (1mg/ml), Crude ethanol extract (100mg/ml), fractions 10mg/ml, Pentobarbitone (100mg/ml) and Adrenalin (1mg/ml).

Extraction method

The pulverized vegetable (1kg) was weighed and macerated in 5L of 96% ethanol for 48h. The whatman No 1 filtrate was dried at 40% and the extract used for analysis.

Fractionation of ethanol extract

The crude ethanol extract was adsorbed on silica gel and was serially fractionated with solvents of increasing polarity n-hexane, chloroform, ethyl acetate and the residual ethanol fraction was washed several times with ethanol solvent and the four fractions were dried and stored for use.

Effect of the crude ethanol extract and its fractions on blood pressure of anaesthetized-cat was done usingthe method of Akah *et al*, (2007). A healthy cat was anaesthetized using intra-peritoneal injection of 50mg/kg (b.w) of pentobarbiton. Incisions were made on left hind limb and on the neck region. The femoral vein, carotid artery and the trachea were all cannulated. The cannula of carotid artery was connected to a blood pressure transducer. The drugs, the crude ethanol extract and its four fractions n-hexane, chloroform, ethyl acetate and the residual ethanol fractions were applied through the femoral vein and their effects on blood pressure monitored through the carotid artery recordings on the blood pressure tranducer. Heights of peaks were measured and used for comparative purposes.

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RESULTS

Table 1: Effect of Crude ethanol extract on blood pressure of anaesthetized cat

Group/drug	Vol.(ml)	Response Mean ±SEM (cm)
Adrenaline	0.1	+ 2.90 ± 0.15a
Acetylcholine	0.1	- 2.93 ± 0.22
Crude	0.5	- *2.20 ± 0.40
Acetylcholine +Atropine	0.1 +0.1	_
Crude + Atropine	0.5 + 0.1	_

Values presented as means ± SEM, *P <0.05, P<0.01 against negative control acetylcholine.

Table 2: Effect of the sub-fractions of crude ethanol extract on the blood pressure of anaesthetized-cat

GROUP/Drug	Vol.(ml)	Response Mean+ SEM (cm)
Adrenalin	0.1	+ 2.90 ± 0.15[a]
Acetylcholine	0.1	-2.93 ± 0.22
n-Hexane extract	0.5	- 2.80 ± 0.03
Chloroform extract	0.5	- 3.77 ± 0.15*
Ethylacetate extract	0.5	- 2.50 ± 0.06
Residual ethanol extract	0.5	- 2.13 ± 0.03*
Acetylcholine + Atropine	0.1 + 0.1	_
n-Hexaneextract + atropine	1.0 + 0.1	_
chloroform + atropine	1.0 + 0.1	_
Ethanol extract + atropine	1.0 + 0.1	2.2
Ethylacetate extract + atropine	1.0 + 0.1	2.5

*P < 0.05, p < 001 against negative control acetylcholine.

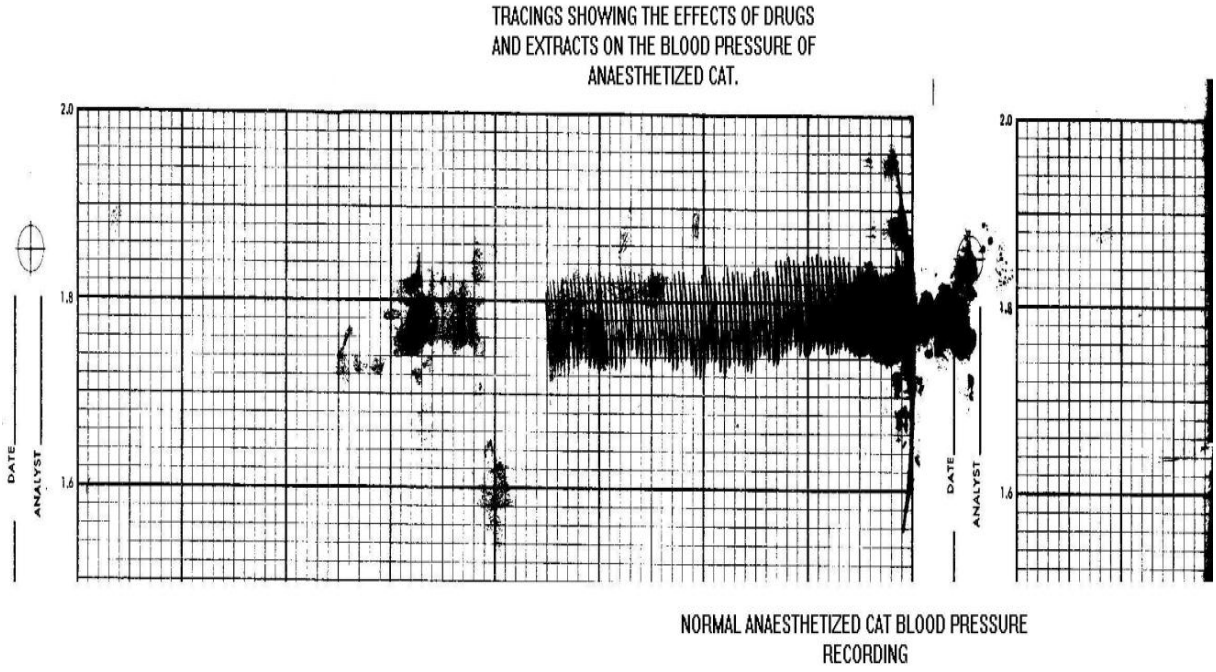

Fig. 1: Normal Blood Pressure of Anaesthetized Cat.

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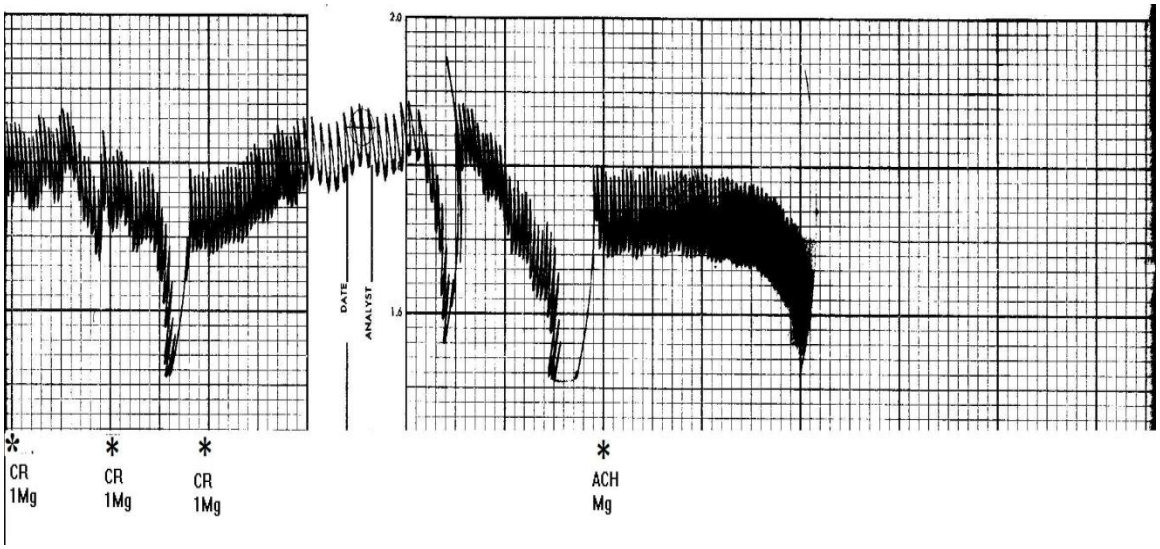

Fig. 2: Blood Pressure Effects of Crude Extract and Acetylcholine.

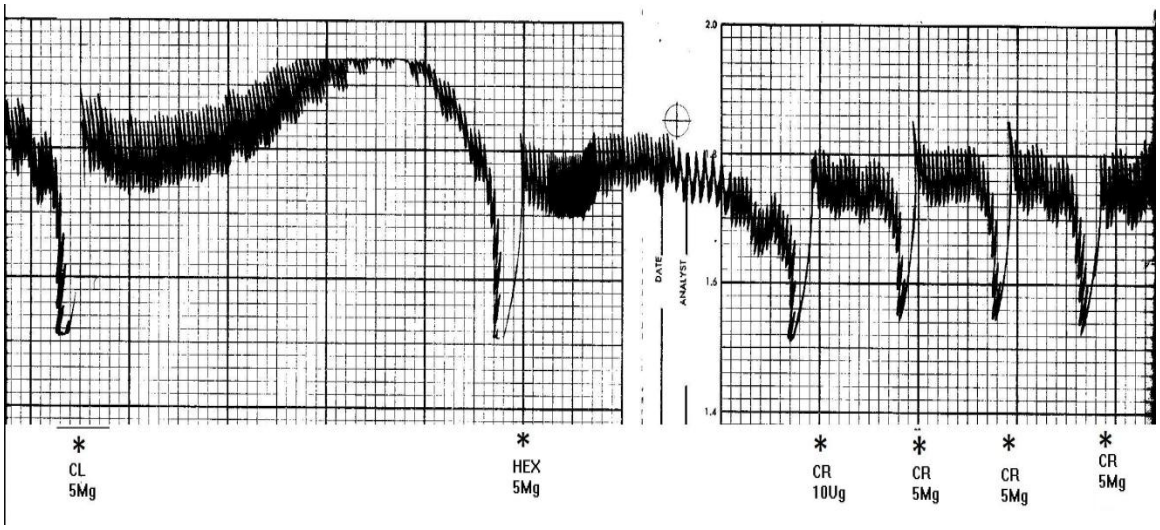

Fig. 3: Blood Pressure of Different Doses of Crude Extract.

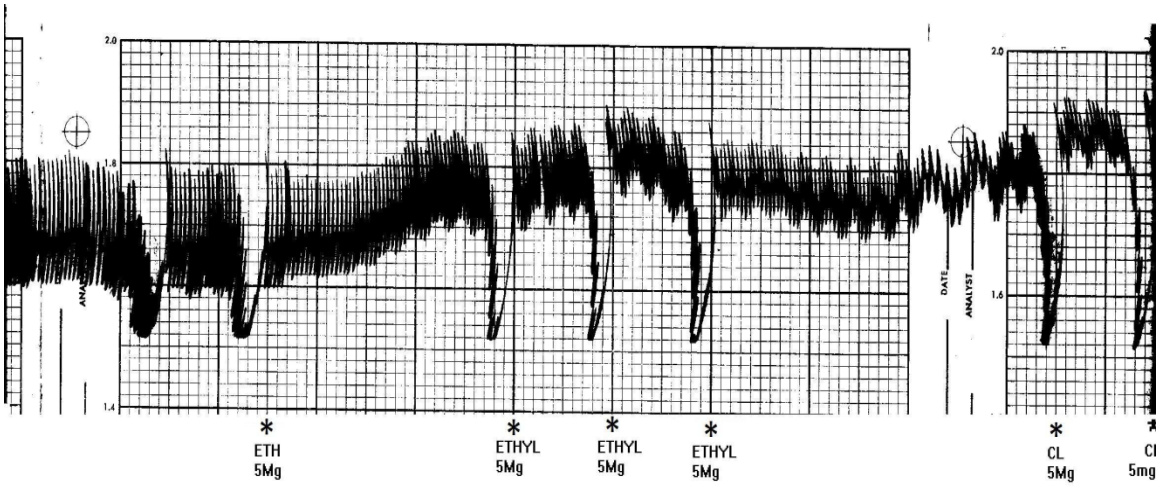

Fig. 4: Blood Pressure Effects of Sub-fractions of the Crude Extract.

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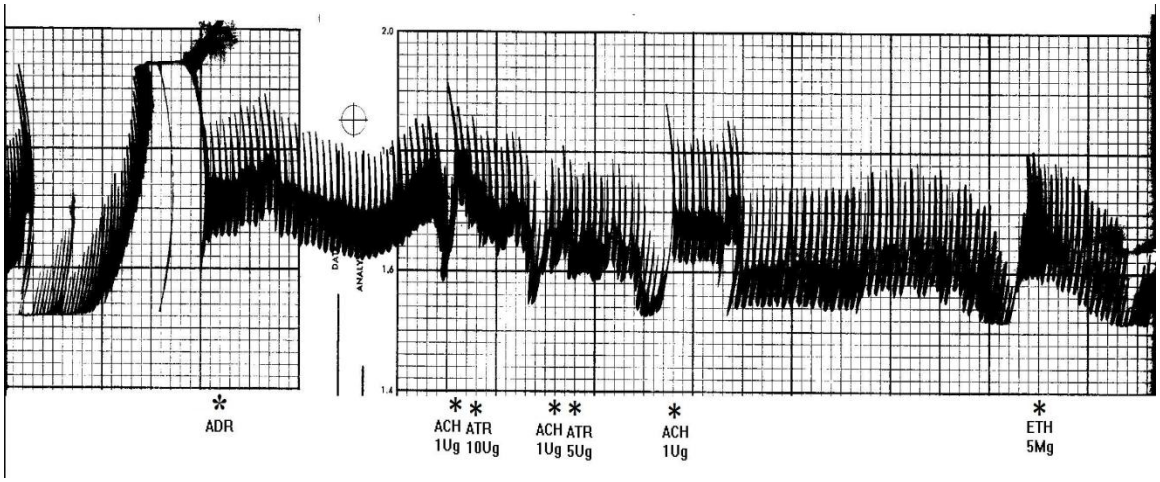

Fig. 5: Blood Pressure Effects of Atropine and Adrenalin.

The results from table 1, 2 and fig.1, 2, 3, 4 and 5 show that acetylcholine lowered blood pressure of anaesthetized cat. Unlike acetylcholine, adrenaline elevated the blood pressure.

In the same animal, the crude ethanol extract greatly lowered the blood pressure like acetylcholine – induced fall in blood pressure. The blood pressure lowering effect of the crude was susceptible to blockage with the muscarinic receptor antagonist, atropine.

In like manner, the four fractions of the crude ethanol extract n-hxane, chloroform, ethyl acetate and the residual ethanol fractions lowered the blood pressure of the cat.

DISCUSSION

Results from this study showed that the crude extract as well as the fractions of the crude hexane, chloroform, ethylacetate and the residual ethanol fractions significantly ($p<0.05$) reduced blood pressure of anaesthetized – cat. They exhibited the vasodepressor effect just as acetylcholine. It is established that parasympathomimetic drugs, like acetylcholine, reduce blood pressure (Shlafer and Marrieb, 1989, Rang *et al*., 1995). These substances act directly on the blood vessels to dilate them, thereby lowering blood pressure. By exhibiting cholinemimetic property of acting directly on the blood vessels to dilate them, these extracts must have operated through the muscarinic antagonist, atropine.

The actions of the crude n-hexane, chloroform, ethylacetate and residual ethanol reduced blood pressure in the order of chloroform > n-hexane >ethylacetate> residual ethanol. It seems to suggest that there were differences in the composition of the fractions and that these components influenced the blood pressure lowering ability. Since chloroform fraction gave a better blood pressure lowering effect, it seems that the phytochemical components had better blood pressure lowering property. Mullen *et al*, (2002) showed that ascorbic acid reduced blood pressure and arterial stiffness in Type 2 diabetes. Flavonoids in fruits, vegetables and red wines are know to inhibit oxidation of low density lipoproteins (LDL-cholesterols) (Kweterovich, 1997, Serafini *et al*, 1998) by scavenging reactive oxygen species (Halliwell, 2002). Other component of fruits and vegetables such as Vit. A and E,

fiber also exhibit inverse relationship to cardiovascular disease (Jingh *et al*., 1992). All of these components in conjunction inhibit cholesterol deposition in the arteries that may result in arteries or scavenge reactive oxygen species thereby preventing oxidative impact that leads to cellular injury. One important component of *Gongronema latifolium* B-Sitosterol (Ezekwe, 2012) is known to competitively inhibit the absorption and deposition of cholesterol in the arteries (Voet and voet, 2011). This makes the vegetable a prospective antilipidemic and anti-hypertensive agents.

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