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EFFECT OF THE CRUDE EXTRACT AND FRACTIONS OF *Occimum gratissimum* ON CAT BLOOD PRESSURE

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ABSTRACT: The fresh leaves of *Ocimum gratissimum* were harvested, macerated with n-hexane, chloroform, ethyl acetate, Acetone and then methanol. The filtrates were concentrated under vacuum using rotary evaporator. The LD_{50} of the crude methanol extract in mice was found to be 1.265g/kg. Phytochemical studies on the methanol extract showed abundance of alkaloids, reducing sugar, flavonoids, glycosides, acidic compounds and saponins. Resins, steroids, tannins and terpenoids were in trace amounts, whereas fats and oil were absent. The anti-hypertensive activity of the crude extract and fractions of *Ocimum gratissimum* were screened using an anaesthetized cat of weight 1.8kg. When acetylcholine (80µg/kg) was administered, it caused cardiac arrest in the animal and there was a sustained decrease in blood pressure translating to a response of 1.2cm. When adrenaline (80µg/kg) was administered, it produced a rise in blood pressure translating to a response of 3.4cm. The extract/fractions on the other hand caused a fall in blood pressure in the intact animal. The *in vitro* studies showed that the extract /fractions antagonized adrenaline induced contractions of the isolated rat vas deferens dose –dependently. From the results, the crude methanol extract and fractions of *Occimum gratissimum* produced a marked fall in blood pressure of anaesthetized cat dose-dependently. The chloroform fraction had the greatest effect (27%), followed by the acetone fraction (25%) and then the n-Hexane fraction (24%). Interaction with atropine shows that the extract/fractions were generally fast - acting as the acetylcholine. The results of the two models employed in the work imply that the fractions possess constituents that have blood pressure lowering action.

INTRODUCTION

Hypertension is an ailment with a high mortality and morbidity rate leading to low human productivity. Many researchers have developed anti-hypertensive agents (Whelton, 1994). Most of these drugs have serious side effects and are not within the reach of the majority of the population. This necessitated the search in the direction of plants for components that possess anti-hypertensive activities. Nigerians use many anti-hypertensive plants (Sofowora, 1993). One of these plants is *Occimum gratissimum.* It has been chosen for further research because there is paucity of information on its presumed anti hypertensive effect.

There is no cure for hypertension and when discovered the treatment is lifelong. It can only be managed by the use of drugs, adequate scientific exercise and relaxation and also by restrictions in salt, calorie and cholesterol intake. This study is aimed at establishing a scientific blueprint for the presumed antihypertensive effect of *Occimum gratissimum* .This may lead to the production of a new, more potent and affordable anti-hypertensive drug with less or no side effect.

Ethnomedical Uses of *Occimum gratissimum*

The leaves contain essential oil rich in thymol and used for treating diarrhoea, Silva *et. al.* 2004, Trease and Evans, 2009). The oil has anti-helmintic properties (Sofowora 1970). Hot decoction of the leaves is taken as tea in the treatment of fever (Irvine, 1962). Pulped leaves mixed with shear butter is used in Ivory Coast against itches and sometimes rubbed on the forehead to stop headache. The juice from the leaves is used as eye drop for conjunctivitis. Hot decoction of the leaves is used to prevent threatened abortion. In Kenya and in Nigeria the hot decoction is inhaled to treat cold and catarrh (Burkill, 1997).The aqueous extract of the leaf is used in the treatment of diarrhoea (Offiah and Chikwendu, 1999). In Nigeria an antimicrobial study using *Proteus, Klebsiella, Escherichia, Salmonella, Staphylococcus, and Shigella* showed zones of inhibitions (Nweze and Eze, 2009).

MATERIALS AND METHOD

The leaves of *Ocimum gratissimum* were plucked from the stems, washed, sliced into small pieces and air-dried. The dried leaves were grounded to moderately coarse particles.

Preparation of Extract / Fraction

The powdered leaves were divided into two portions of 300g each. The first portion was macerated with 2.5 litres of methanol for 48 hours. The Whole Methanol Extract (WME) was concentrated using rotary evaporator. The second portion was successively extracted with n-hexane, chloroform, ethyl acetate, acetone and then methanol. The extracts were concentrated with rotary evaporator to obtain the n-hexane, chloroform, ethyl acetate, acetone, and methanol fractions. The acute toxicity test was determined (Lorke, 1983). Phytochemical screening was also done (Trease and Evans, 2009, Harbone, 1998).

Screening for Anti-Hypertensive Effect Using Anaesthetized Cat

A cat of 1.8kg was anaesthetized with pentobarbitone injection (50mg/kg) given intraperitoneally. Incisions were made on the neck region and left hind limb and cannulas were inserted in the femoral vein, the carotid artery and the trachea. 0.2mls of Heparin, the extracts and the drugs were given to the cat through the femoral vein and washed with normal saline until the end of the experiment. The carotid artery cannula was connected to a blood pressure transducer, through which changes in blood pressure were recorded on Ugo Basile double channel recorder.

Isolated Tissue *(In vitro)* Experiment

An adult male rat was sacrificed by stunning and excision of the throat. The abdomen was opened and the gut lifted aside, a thread was tied at both ends of the vas deferens. The vas deferens was cut just above the epididymis and also at the point where it joins the urethra. The preparation was mounted in Krebs's solution and was aerated with a mixture of oxygen (95%) and carbon dioxide (5%). The other end of the thread was attached to the lever. The dose-response effect of adrenaline was established. The extract was administered, allowed for 2 minutes before various doses of adrenaline were added. The effect on the normal stimulation by adrenaline recorded. The normal 30 second cycle was observed with the adrenaline experiments. The tissue was allowed to equilibrate for 30 minutes before any drug was introduced.

RESULT

Phytochemical Analysis

The result of the phytochemical screening showed that *Ocimum gratissimum* contain alkaloid, glycoside, reducing Sugar, Carbohydrate, saponin, flavonoid, acidic compounds, terpenoids, steroids, resins and tannins

Table 1: Effect of adrenaline and acetylcholine on Vas Deferens

S/NO.	ADRENALINE Concentration (µg)	Response (cm)	ACETYLCHOLINE Concentration(µg)	Response (cm)
1	10	1.3	10	3.8
2	20	2.0	20	2.7
3	40	2.5	40	1.9
4	80	3.4	80	1.2

Table 2: Action of adrenaline and crude extract/fractions on Vas Deferens 1 And 2.

		VAS DEFERENS 1			VAS DEFERENS 2		
S/N		E/F (mg)	Adr. (ug)	Res. (cm)	E/F (mg)	Adr. (ug)	Res. (cm)
1	Ethylacetate	2	20	0.3	2	10	0.4
	Fraction	2	40	0.4	4	10	0.4
	+ Adrenaline	2	80	1.0	8	10	0.1
2	Chloroform Fraction	2	20	0.5	2	10	0.4
	+ Adrenaline	2	40	0.8	4	10	0.3
		2	80	1.1	8	10	0.3
3	Acetone Fraction	2	20	0.3	2	10	0.4
	+ Adrenaline	2	40	0.7	4	10	0.2
		2	80	1.2	8	10	0.3
4	n-hexane Fraction	2	20	1.1	2	10	0.4
	+ Adrenaline	2	40	1.35	4	10	0.5
		2	80	1.7	8	10	0.4
5	Methanol Fraction	2	20	1.5	2	10	0.3
	+ Adrenaline	2	40	1.8	4	10	0.3
		2	80	2.3	8	10	0.1
6	Crude Extract +	2	20	0.8	2	10	0.5
	Adrenaline	2	40	0.1	4	10	0.3
		2	80	1.3	8	10	0.25

Note: In vas deferens 1, dose of extract/ fraction is fixed while that of adrenaline varies but in vas deferens 2 the reverse is the case. (E/F = Extract/Fraction, Adr. = Adrenaline, Res. = Response).

DISCUSSION

The LD_{50} of the crude extract was calculated as 1. 265 g/kg. The anaesthetized cat is an intact animal and so has α_1, α_2, β_1 and β_2 receptor subtypes. When acetylcholine was administered there was a sustained decrease in blood pressure. The sustained decrease may be due to the amount of the drug that was introduced. The intravenous injection of small dose of acetylcholine produces an evanescent fall in blood pressure owing to generalized vasodilatation accompanied usually by reflex tachycardia (Sabyasachi 2007, Shen 2008 Saganuwan 2009, Saliu *et. al.*, 2011)

When adrenaline was administered, it produced a rise in blood pressure. Adrenaline is a very potent vasoconstrictor and cardiac stimulant. The rise in systolic blood pressure that occurs after adrenaline release or administration is caused by its positive ionotropic and chronotropic actions (predominantly β_1 receptors) and the vasoconstriction in many vascular beds (α receptors). Adrenaline also activates β_2 receptors in some vessels (e.g. skeletal muscle, blood vessels), leading to their dilation. Consequently, total peripheral resistance may actually fall, explaining the fall in diastolic pressure that is sometimes seen with adrenaline injection (Sabyasachi, 2007, Saganuwan, 2009, Saliu etal, 2011)

The extract/fractions on the other hand caused a fall in blood pressure in the intact animal probably by α_1-blockage. This α_1-blockade action results in inhibition of the coupling of adrenaline receptors to phospholipase C. This obstructs the series of reaction mediated by phospholipase C leading to decrease in systolic concentration of Ca^{2+}. The total peripheral vascular resistance decreases as a result of peripheral vascular vasodilation.

The *invitro* studies showed that the extract /fractions antagonized adrenaline induced contractions of the isolated rat vas deferens dose dependently with chloroform and acetone fraction, having the greatest effect. The vas deferens has dense α-receptor distribution. α-receptors mediate the excitatory effects of sympathomimetic amines (except in the smooth muscle of the gut). Adrenaline induces contraction on the isolated rat vas deferens by stimulation of α_1 receptor. This antagonism of adrenaline induced contraction of the rat vas deferens by the extract is a good evidence of α_1-blockage. Since sympathetic stimulation of α_1–receptors results in vasoconstriction and increase in peripheral vascular resistance; the antagonism of adrenaline induced contraction at the post synaptic effector cells in arteriolar and venous bed would result in vasodilatation and decrease in peripheral vascular resistance. As a result of this decrease the main haemodynamics abnormality is corrected and a decrease in blood pressure follows (Sabyasachi 2007, Shen 2008, Saganuwan, 2009, and Saliu *etal,* 2011).

CONCLUSION

From the results, the crude methanol extract and fractions of *Occimum gratissimum* produced a marked fall in blood pressure of anaesthetized cat dose-dependently. The order of activity of fractions was chloroform > acetone > n-Hexane. Based on the above results, the extract/fractions of *Ocimum gratissimum* can therefore be used as an anti-hypertensive. The extract exerts its anti-hypertensive effect by decreasing the pulmonary vascular resistance due to α_1- blocking activity in vascular bed.

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