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THE ANTIHYPERTENSIVE EFFECT OF N-HEXANE AND AQUEOUS ETHANOLIC EXTRACTS OF THE SCHLEROTIUM OF *PLEUROTUS TUBER-REGIUM* IN SODIUM CHLORIDE INDUCED ALBINO RATS

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Abstract

Antihypertensives are a class of drugs that are used to treat hypertension (high blood pressure). Its therapy seeks to prevent the complications of high blood pressure, such as stroke and myocardial infarction. The sclerotium of pleurotus tuber-regium is used in folkloric medicine in the treatment of high blood pressure. This study was designed to determine the in vivo antihypertensive effect of nhexane and 50% aqueous ethanolic extract of the sclerotium of pleurotus tuber-regium in high-salt (NaCl) diet-induced hypertensive rats. The effect of the extracts showed a non dose-dependent antihypertensive effect at (1000µg/kg, 4000µg/kg, 5000µg/kg). The Systolic/Diastolic blood pressure and pulse pressure of the n-hexane

extract and 50% aqueous ethanolic extracts were 118.31mmHg/100.34 mmHg with the pulse pressure of 12.94 and 125.11/110.25mmHg with PP of 14.86 at 1000µg/kg, 120.25/107.31mmHg with PP of 17.97 and 130.09/117.71mmHg with PP of 12.38 at 5000µg/kg and 125.99/112.61mmHg and 132.24/115.99mmHg with PP of 13.38/16.25 at 10000µg/kg respectively. The standard test drug Nifedipine gave the SBP/DBP of 105.17/87.72mmHg with the PP of 17.45, Hydrochlorothiazide (HCT) gave 138.28/126.81mmHg with PP of 11.47, Telmisartan gave 174.94/141.29mmHg with the PP of 33.65 and Enalapril gave 128.99/115.08mmHg with PP of 13.91. The antihypertensive activity of n-hexane is similar to that of Nifedipine and the activity of 50% aqueous ethanol with enalapril. Thus, they can be said to have the same mechanism of action. This implies that the extract possess anti-hypertensive properties and could be employed for drug development. .

INTRODUCTION

Globally, cardiovascular diseases account for approximately 17 million deaths a year (W.H.O 2008), nearly one third of the total of these complications is hypertension which accounts for 9.4 million deaths worldwide every year (Lim SS *et al*., 2010). Hypertension, also known as high blood pressure (HBP), is a long term medical condition in which the blood pressure in the arteries is persistently elevated (*Naish and Jeannette, 2014*). High blood pressure usually does not cause symptoms. Long term high blood pressure, however, is a major risk factor for coronary artery disease, stroke, heart failure, peripheral vascular disease, vision loss, and chronic kidney disease (*Lackland et al., 2015*).

Antihypertensives are a class of drugs that are used to treat hypertension (high blood pressure). Antihypertensive therapy seeks to prevent the complications of high blood pressure, such as stroke and myocardial infarction. Evidence suggests that reduction of the blood pressure by 5 mmHg can decrease the risk of stroke by 34%, of ischaemic heart disease by 21%, and reduce the likelihood of dementia, heart failure, and mortality from cardiovascular disease *(Law M. et al., 2003)*. There are many classes of antihypertensives, which lower blood pressure by different mechanisms.

Among the most important and most widely used drugs are thiazide diuretics, calcium channel blockers, Angiotensin-converting enzyme inhibitors (ACE), angiotensin II receptor antagonists (ARBs), and beta blockers.

In nature, sclerotia typically form in response to adverse growing conditions as a method of carrying the life of the fungus through difficult conditions. When the growth medium dries out or available nutrients are used up, the fungus responds by forming a sclerotium (Olukoya and Okogbo, 1990). It is spherical to ovoid in shape and can be quite large (up to 30cm or 11.8 inches) or larger in diameter (Oso, 1997). The sclerotium of *Pleurotus tuber-regium* contains sufficient quantities of these minerals to meet the nutritional requirement of human beings and animals. In the Nigerian indigenous languages it is called "Katala" in (Hausa), "ike usu/ero usu" in (Igbo), "Awu" in Igala and "ohu" in Yoruba. The fiber-rich tuber or sclerotium is used as a soup thickener because of its ability to swell in water and add bulk. It is used in cooking egusi (melon) soup. Yongabi (2004) confirmed that the sclerotium of *P. tuber-regium* is a good coagulant and a disinfectant which can be used in natural and waste water purification.It plays important roles in the maintenance of normal glucose tolerance and in the release of Insulin from beta cells of the Islets of langerhans (Choudlang and Bandyopadhyay, 1999). The Sclerotia of *Pleurotus tuberregium* are a rich source of proteins, fibers and carbohydrates, and are potential source of nutraceuticals, hence its use in Chinese folklore recipes for the treatment of coughs and asthma (Chengua *et al*., 2000).

Materials and Methods

Collection and identification of the plant material.

The sclerotia of *Pleurotus tuber- regium* were purchased from Oyingbo market in Lagos State, Nigeria, between February and March 2015 and taxonomically identified by Mr.

Oyebanji of the Department of Botany, University of Lagos, with voucher number "LUH:

003M". The sclerotia were washed several times to remove dirt and debris; cut into smaller pieces and air-dried. The dried sclerotia were then milled into powdery form and stored for further use.

Preparation of extract

Approximately 6kg (equivalent to 6000g) of powdered sclerotium was weighed and macerated in 25 litres of n-hexane to be de-fatted for 3 days with daily agitation after which it was filtered. The filterate was collected in a beaker, and was concentrated using rotary evaporator**.** The marc collected after filtration of the n-hexane extract was air dried and the above process was repeated using aqueous ethanol (50%). The filtrates were collected in a beaker, and concentrated using rotary evaporator and oven dried at a temperature of 40^{o}C to reduce the moisture content and the extracts were used for the *in vivo* antihypertensive study.

Antihypertensive evaluation

Animals used

The antihypertensive activity of the extract was carried out on 80 female albino rats aged 5-6 weeks and weighed 70-90 g prior to the experiment. The animals were housed in standard environmental conditions under a 12/12 hour light/dark natural cycle in the animal house of the Faculty of Pharmacy, in the Lagos University Teaching Hospital premises. All animals had free access to standard diet and tap water for seven days for acclimatization and equilibration on the standard growers feed. Each Albino rat was weighed prior to any form of work.

Experimental design

The antihypertensive activity of the n-hexane and ethanolic extract**s** of sclerotium of *P. tuber-regium* were evaluated using salt-induced hypertension model previously described by Sofola *et al* and Balogun *et al.* The rats were placed on high-sodium diet with 8% NaCl for a period of 6 weeks. Rats were randomly divided into groups of five rats each for the different extracts respectively and daily treated for three consecutive

weeks. Animals in group one(1) received no salt diet; they were given growers feed and water. Animals of group two(2) were given the salt diet. After six weeks of 8% salt-diet, the nhexane and 50% aqueous ethanol extracts of various doses (1000, 4000 and 10000) µg/kg were administered orally to groups two to group five (2-4) respectively while groups five, six, seven and eight received the standard drugs Nifedipine (10 mg/kg), Hydrochlorothiazide (25mg/kg), Enalapril (5mg/kg) and Telmisartan (40mg/kg) which were given orally. At the end of the investigation period, blood pressure, pulse pressure, mean arterial blood pressure and heart rate of the rats were measured as described by (Balogun *et al*., 2016)

Measurement of blood pressure and heart rate

Invasive blood pressure measurement was carried out via arterial cannulation. The rats were anaesthetized with a solution of 25% (w/v) urethane and 1% (w/v) α-chloralose injected intraperitoneally at a dose of 5 ml/kg body weight. The anaesthetized rat was placed on its back on the operating table, the limbs were fastened to the table, and the trachea was exposed and cannulated. The blood pressure measurements were obtained by cannulation of one of the carotid artery. A polyethylene cannula filled with 1% heparinised saline was inserted into the artery, tied in place, and connected via a pressure transducer (model SP 844, Physiological Pressure Transducer. AD Instruments) that was attached through MLAC11 Grass adapter cable to a computerized data acquisition system with LabChart-7 pro software (Power Lab-4/24T, model MLT844/P; AD Instruments Pty Ltd., Castle Hill, Australia). The LabChart-7 Pro software computes the HR by applying the cyclic measurement function, which is a channel calculation that analyzes periodic blood pressure waveforms in real time. Data of the detected cycles **were** displayed as a continuous data trace for HR in another channel of the data acquisition system. Recordings were taken at a sampling frequency of 5/seconds.

RESULTS AND DISCUSSION

Antihypertensive activity carried out on albino rats after induction with 8% NaCl for 3 weeks

Table 1: Result obtained from the measurement of blood pressure three weeks after salt (NaCl) diet.

Key: SBP = Systolic blood pressure. DBP = Diastolic blood pressure. PP = Pulse pressure. MABP = Mean arterial blood pressure. HR = Heart rate.

Animal	SBP	DBP	PP	MABP	HR
Rat 1	126.07	114.89	11.18	118.6	342
Rat 2	126.25	106.66	19.59	113.2	330
Rat 3	143.30	126.66	16.54	132.2	420
Rat 4	138.17	125.77	12.42	129.9	432
Average Values	133.45	118.50	14.93	123.48	381

Table 2: Result obtained from the measurement of blood pressure obtained one week after treatment with the n-hexane extract of sclerotium of *P. tuber-regium* and the standard antihypertensive drugs.

Animal	*SBP*	*DBP*	*PP*	*MABP*	*HR*
1000ug/kg	*118.31*	*100.34*	*12.94*	*111.6*	*372*
5000ug/kg	*120.25*	*107.31*	*17.97*	*106.3*	*372*
10000ug/kg	125.99	112.61	13.38	117.1	408
Nifedipine	105.17	87.72	17.45	93.54	312
HCT	138.28	126.81	11.47	130.6	348
Telmisartan	174.94	141.29	33.65	152.5	372
Enalapril	128.99	115.08	13.91	119.7	324
Untreated	138.17	118.84	19.33	125.3	360
Average Values	131.26	113.75	17.51	119.54	358.5

Key: SBP-Systolic blood pressure, DBP-Diastolic blood pressure, PP-Pulse pressure, MABP-Mean arterial blood pressure, HR-Heart rate.

Table 4: Showing results of blood pressure monitoring after treatment with 50 % aqueous ethanolic extract of sclerotium from *P. tuber-regium*

Animal	SBP	DBP	PP	MABP	HR	RPP
1000ug/kg	125.11	110.25	14.86	115.2	432	54047.52
5000ug/kg	130.09	117.71	12.38	121.8	444	57759.96
10000ug/kg	132.24	115.99	16.25	121.4	420	55540.8
Enalapril	128.99	115.08	13.91	119.7	324	41792.76
Nifedipine	105.17	87.72	17.45	93.54	312	32813.04
HCT	138.28	126.81	11.47	130.6	348	48121.44
UNTREATED	138.17	118.84	19.33	125.3	360	49741.2
Average Values	112.26	88.09	13.21	103.44	330	42477.09

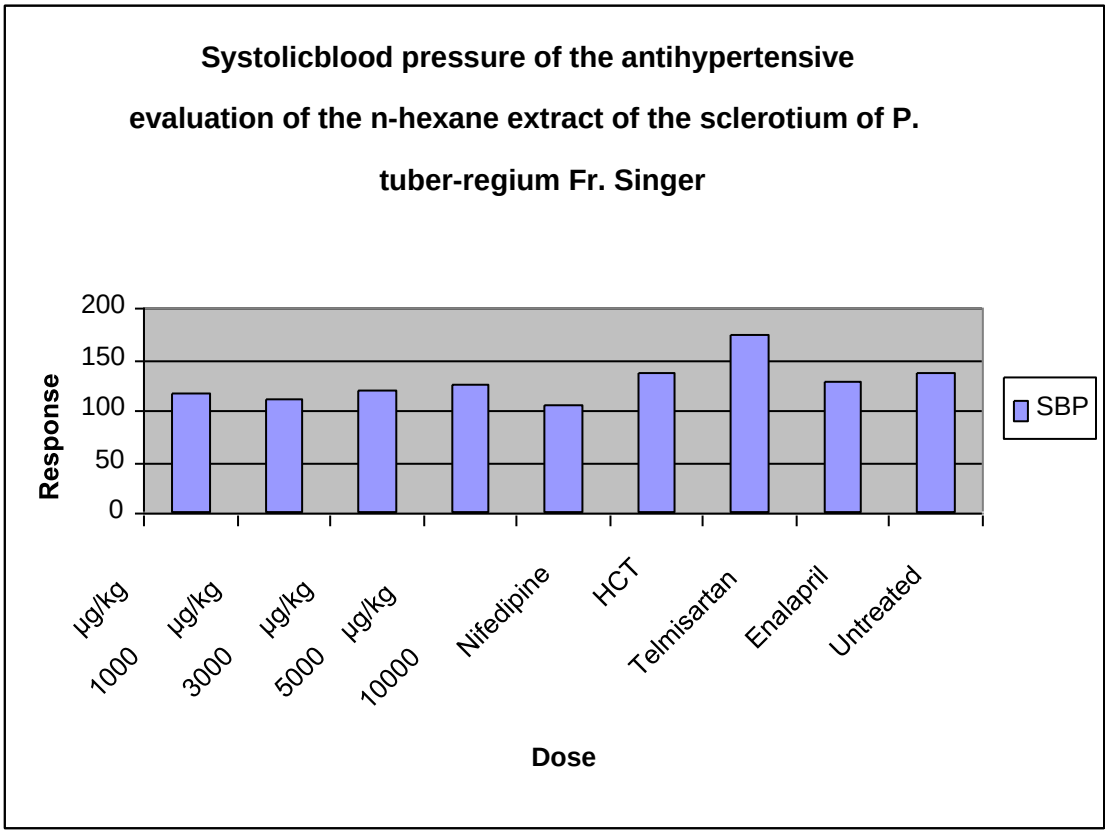

Figure 1: A chart showing the systolic blood pressure of the antihypertensive evaluation of the nhexane extract of the sclerotium of *Pleurotus tuber-regium* in comparison with the standard drugs.

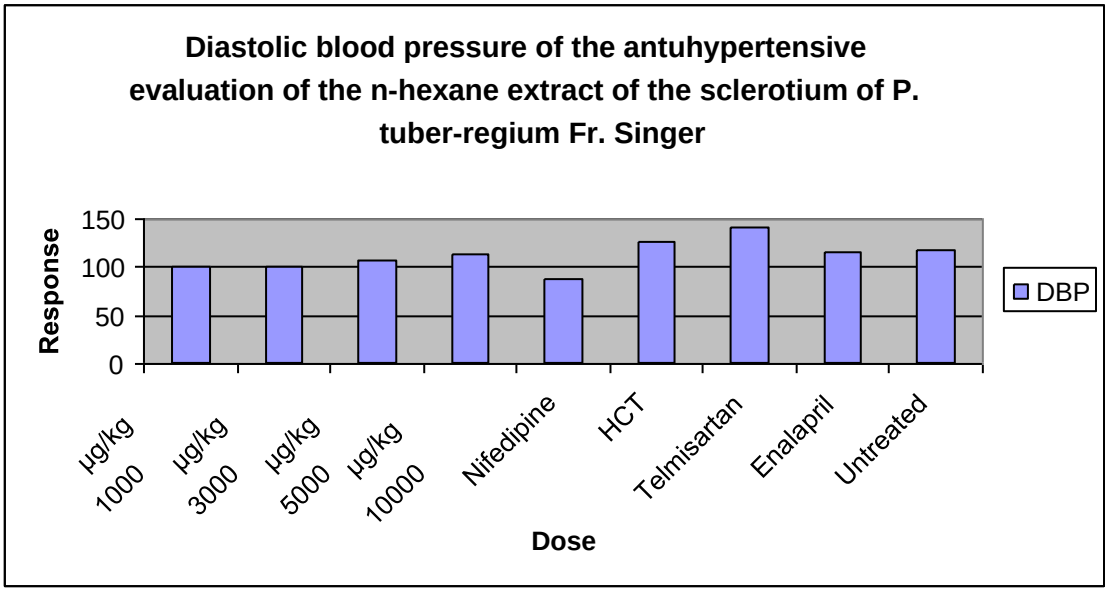

Figure 2: A chart showing the diastolic blood pressure of the antihypertensive evaluation of the nhexane extract of the sclerotium of *Pleurotus tuber-regium* in comparison with the standard drugs.

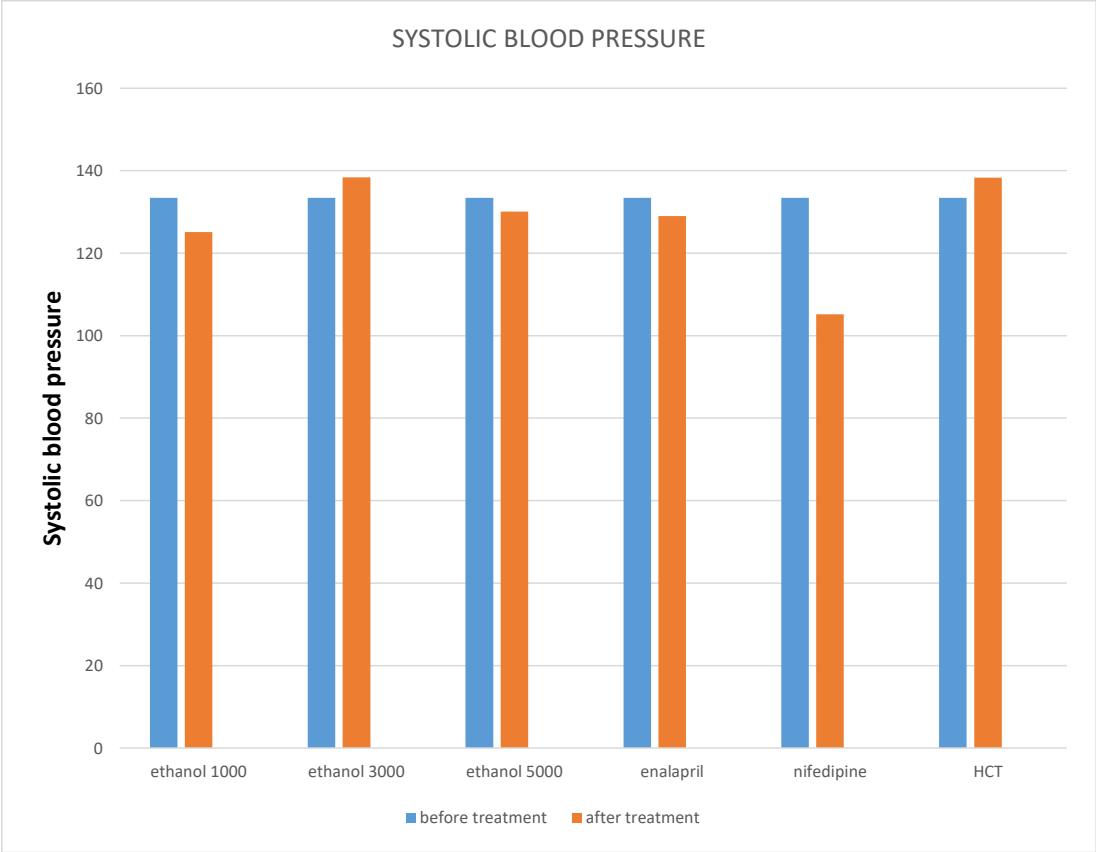

Figure 6 above illustrates the effect of treatment with 50% aqueous ethanolic extract and various antihypertensive agents on the systolic blood pressure. Ethanol 1000ug and ethanol 5000ug extract showed significant post-treatment reduction in systolic blood pressure

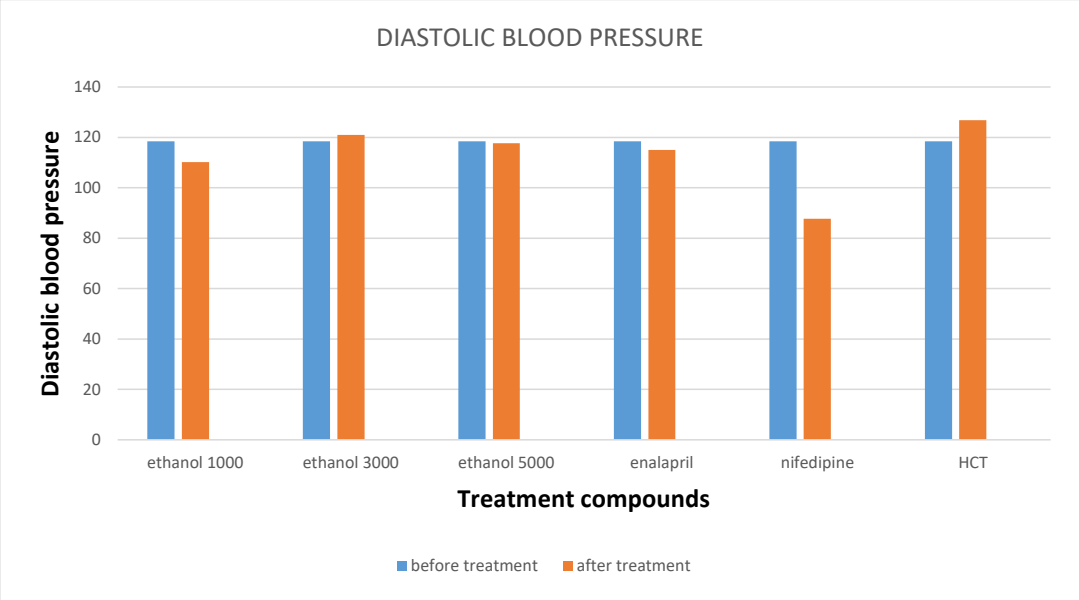

Figure 7 above illustrates the effect of treatment with 50% aqueous ethanolic extract and standard antihypertensive agents on the diastolic blood pressure.

In this study, the anti-hypertensive analysis of the n-hexane and 50% aqueous ethanolic extracts of the sclerotium of *P. tuber-regium* were conducted in the hypertensive inducement model of NaCl. Over the past century, salt has been the subject of intense scientific research related to blood pressure elevation and cardiovascular mortalities. The mechanism by which NaCl causes hypertension is through sodium ion, one of this is, when smooth muscles contract, vessels around constrict leading to an increased peripheral resistance resulting in an increase in blood pressure. Another mechanism by which sodium works is through the accumulation of water; that is, fluid retention. This fluid retention increases preload and after load which increases cardiac output leading to an increase in blood pressure. The *in vivo* antihypertensive activity of *n*-hexane extract at a dose of 1000µg/kg, the systolic and diastolic blood pressure was observed to be reduced by

15.14/18.16mmHg with pulse pressure of 12.94, at 5000µg/kg it was 13.2/11.19mmHg with pulse pressure of 17.97 and at 10000µg/kg it was 7.46/5.89mmHg with pulse pressure of 13.38. While the 50% aqueous ethanol extract from sclerotium of *Pleurotus tuberregium* at concentrations of 1000µg the Mean of the SBP reduced by 8.34mmHg and the Mean of the DBP reduced by 8.25mmHg and at 5000µg the Mean of the SBP was reduced by 3.36mmHg and the Mean of the DBP was reduced by 0.78mmHg thus, leaving the 1000ug dose of the 50% aqueous ethanolic extract with the highest antihypertensive activity at 125.11/110.25mmHg this was found to be similar to the standard test drugs enalapril which reduced the mean of SBP/DBP to
128.99/115.08mmHg. The standard drugs Enalapril reduced the systolic/diastolic blood pressure by 4.46/3.42mmHg, Nifedipine reduced the mean of SBP/DBP by 28.45/30.77mmHg, Termisaltan.
Nifedipine had the highest antihypertensive activity among the standard test drugs with blood pressure of 105.17/87.72mmHg.
Evidence suggests that reduction of blood pressure by 5mmHg can decrease the risk of stroke by 34%, ischaemic heart disease by 21% and reduce the likelihood of dementia, heart failure and mortality from cardiovascular diseases (Law.M *et al.,* 2003). From the results obtained it was observed that n-hexane extract had a higher antihypertensive activity than the 50% ethanolic extract.

CONCLUSION

Both extracts constitutes secondary metabolites which are responsible for its antihypertensive activity. Although they both showed antihypertensive activities, the n-hexane extract was more antihypertensive with a dose dependent activity.

RECOMMENDATION

The n-hexane and the 50% aqueous ethanolic extracts of the sclerotium of *P. tuber-regium* most likely contain certain active principles that are responsible for their antihypertensive activities. Further studies are

required to isolate these phytochemical constituents and elucidate their possible mechanism of action.

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