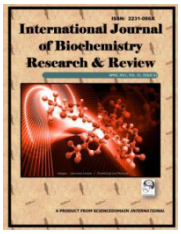

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Antihypertensive Effect of Methanol Extract of *Napoleona imperialis* (p. beauv) in Adrenaline Induced Hypertensive Albino Rats

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ABSTRACT

The aim of this study was to investigate the antihypertensive effect of the methanol extract of the leaf of *Napoleona imperialis* (p. beauv) in adrenaline induced hypertensive rats (AlHR) and compared with those of Propanolol in a cross over design. *Napoleona imperialis* leaf extract and the commercial drug (Propanolol) were administered orally and through intraperitoneal (I.P) route respectively for one week. Effect of the extract on different biochemical parameters such as blood glucose, serum triglycerides, serum cholesterol, serum creatine phosphokinase, lactate dehydrogenase, pulse rate and body weight were measured utilizing standard methods. *Napoleona imperialis* leaf extract at the doses of 26mg/130±17g and 52mg/130±17g of body weight were given orally every morning during treatment to show the effect of dose concentration. Propanolol at a dose of 0.084mg/130±17g of body weight was injected into rats according to its pharmacokinetic parameters. The extract administered rats had significantly lowered serum lactate dehydrogenase, creatine phosphokinase and pulse rate compared to the non-extract administered AlHR group ($P<0.05$). Blood glucose, serum triglycerides and serum cholesterol were not lowered and significantly different ($P<0.05$) when compared with the control. These results suggest that oral administration of methanol extract of *Napoleona imperialis* may be useful for prevention and treatment of hypertension.

Keywords: Napoleona imperialis, antihypertension, creatine phosphate kinase, lactate dehydrogenase and hypertensive;

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1. INTRODUCTION

Hypertension or high blood pressure is a common disorder which if not effectively treated results in a greatly increased probability of coronary thrombosis, stroke, and renal failure (Rang et al., 1999). Circulatory system diseases such as hypertensive, arteriosclerosis, and heart disease are especially prevalent in developed countries, with increasing numbers of people showing symptoms of hypertension or prehypertension (Goldblatt et al., 1934).

Hypertension is becoming a household disease now in developing countries, such as Nigeria. Hypertension can be caused by many factors, including increases in the volume of body fluid, resistance of the blood vessels, and other factors that elevate blood pressure (Takahashi and Smithies, 2004). These factors induce abnormal increases in blood pressure, either alone or in combination. Antihypertensive effects have been noted with various food and natural products (Fatehi et al., 2005; Sato et al.,2002; Amos et al., 2003).

Treatment of hypertension reduces cardiovascular risk, and this has been a major focus of campaigns aimed at reducing cardiovascular mortality and morbidity (Elsik and Krum, 2007). A number of international guidelines suggest that blood pressure should be reduced at least to below 160/90 mmH_g to normalize cardiovascular risk in patients with hypertension. In patient at higher baseline risk of cardiovascular disease, for example those with diabetes, the recommendations are that the target blood pressure should be substantially lower than 130/85 mmH_g. This recommendation is based on the view that the absolute risk of a cardiovascular event in these patients is much greater, and therefore, the absolute benefit of treatment is larger (Elsik and Krum, 2007).

Because of high incidence and morbidity, various drugs and regimes have been advocated for control of hypertension. Many new drugs have been introduced which may demonstrate better efficacy but posses side effects. Recently attention has been drawn or focused towards herbal and mineral preparations which are traditionally used as potential therapeutic agents in the prevention and management of cardiovascular diseases (Bhatt et al., 1998).

Napoleona imperialis is a tree or shrub that seldom grows above 6m with large leaves. It belongs to the family *Lecythidaceae* which is a small tropical plant that grows in all regions of Nigeria and other parts of West Africa (Dalziel, 1955; Hutchinson and Dalziel, 1958).

Though *Napoleona imperialis* is one of the lesser known plants, its economic importance has partially been reported by Dalziel, 1955 and Irvine, 1961). These include the use of its fruits sugary pulp as dessert, the roots for medicinal purposes and the twigs as chewing sticks.

The methanol extract of *Napoleona imperialis* has shown antibacterial and wound healing properties. It showed more than 90% wound healing activity. Its antibacterial properties was studied against eleven chemical isolates (4 strains of *staphylococcus aureus*, 2 strains of *Escherichia coli*, *Shigellia sp* (1 strains), *Pseudomonas aeruginosa* (1 strain) and 3 strains of *Proteus sp.* (Emuelosi et al., 2005). Investigation also has shown that the seeds of *Napoleona imperialis* are rich sources of commercial hemolytic saponins and feed ingredient (Ukpabi and Ukpabi, 2005). Proximate and phytochemical composition of *Napoleona imperialis* showed the presence of phytates, tannins alkaloids, saponins, and metabolizable energy value (Uchegbu et al., 2005).

Generally, the cytosolic enzymes lactate dehydrogenase (LDH) and creatine phosphate kinase (CPK) as well as lactate in coronary effluent are important biomarkers for myocardial ischemia in hypertension (Hropot et al., 2003). There are no reports in the literature, to the best of our knowledge on the hypotensive effect of *Napoleona imperialis* leaf extract. Therefore, the objective of this present study was to investigate the hypotensive effect of *Napoleona imperialis* methanol leaf extract in adrenaline-induced hypertensive rats using serum LDH, CPK and other biochemical parameters as biomarkers.

2. MATERIALS AND METHODS

2.1 PLANT MATERIAL COLLECTION

Fresh leaves of *Napoleona imperialis* were collected from the demonstration farm of the faculty of Agriculture, Kogi state University and identified in the Department of Biological sciences (Botany unit), Kogi state University, Anyigba, Nigeria. The fresh leaves were air-dried for three weeks and pulverized into fine powder using a mortar and pestle.

2.2 PREPARATION OF PLANT EXTRACT

The powdered sample (200g) was extracted in 500 ml of methanol for 72 hours. The extract was filtered using a vacuum pump and concentrated by removing the solvent completely using a water bath.

2.3 ANIMAL MODEL

Twenty (20) albino rats (*Rattus novergicus*) were purchased from the animal farm of the Department or Veterinary Parasitology Sciences, University of Nigeria, Nsukka. Rats were allowed to acclimatize for two weeks with access to clean water and standard animal feeds at the experimental site. A cycle of light and dark (12 hours each) and a temperature of 27 ± 2^0C were maintained in the room. After the period of acclimatization, rats were anaesthetized with diethyl ether and 0.1 ml of adrenaline was injected into rats by intraperitoneal (I.P) injection using a 1 ml disposable syringe for five consecutive days to induce hypertension.

To confirm the induction of hypertension, pulse rate, body weight, serum cholesterol, blood glucose serum triglycerides, serum lactate dehydrogenase and serum creatine phosphokinase levels were measured and compared with that of the control rats that received only normal saline and the standard drug (Propanolol).

2.4 PREPARATION OF THE DOSE OF PLANT EXTRACT

Napoleona imperialis leaf extract was given as 200 and 400 mg/kg body weight of rats. The average body weight of the hypertensive rats was 130±17g. Thus the daily single dose of *Napoleona imperialis* leaf extract was 26mg/130±17g body weight of rats for 200 mg/kg and 52 mg/130±17g body weight of rats for the 400 mg/kg to compare the effect of dose concentration of the extract. The extract was dissolved in distilled water.

2.5 DOSE PREPARATION OF ANTIHYPERTENSIVE DRUG

The daily dose of Propanolol for human is 45 mg/70kg. The average body weight of hypertensive rats was measured 130±17g. So the daily dose of Propanolol was 0.084mg/130±17g body weight of rats.

2.6 ANIMAL GROUPINGS/TREATMENT

Twenty (20) albino rats were divided into five groups of four rats each.

Group 1: Control rats that received only normal saline
Group 2: Untreated adrenaline induced hypertensive rats (AIHR)
Group 3: *Napoleona imperialis* treated adrenaline induced hypertensive rats (26 mg/130±17g b.w.)
Group 4: Positive control group treated adrenaline induced hypertensive rats with propanolol.
Group 5: *Napoleona imperialis* treated AIHR (52mg/130±17g b.w.).

Napoleona imperialis leaf extract was given orally while the commercial drug (Propanolol) was administered through intra peritoneal (I.P) route once daily for one week.

2.7 DETERMINATION OF PULSE RATE

The pulse rates of the animals were measured according to the method described by Rod, 2008. The femoral artery in the groin on the medial aspect of the femur of the hind leg was used. The rats were restrained and once settled, the pulses were taken by placing finger over the femoral artery. The pulse rate was counted for one minute using a stop watch.

2.8 DETERMINATION OF SERUM CREATINE PHOSPHOKINASE ACTIVITY

The activity of serum creatine phosphokinase was determined after one week of treatment according to the method described by Szasz et al.,1976 (Randox C.K. 110 kit was utilized for the quantitative in vitro determination of the enzyme in serum). Twenty 20 ml of the sample was pipetted into a test tube and mixed with 1 ml of the reagent (Randox CK 110 kit). The mixture was left to incubate at 25^0C for 3 minutes and the initial absorbance was read. The absorbance was taken again after 1, 2 and 3 minutes simultaneously at 340nm using an Agilent 8453 spectrophotometer. The creatine phosphokinase activity was calculated using the formula:
U/L = 4127xDA 340nm/min
Where DA = Change in absorbance.

2.9 DETERMINATION OF SERUM LACTATE DEHYDROGENASE

The serum lactate dehydrogenase activity was determined after one week of treatment according to the method described by Weishaar, 1975. A portion (2 ml) of the sample was pipetted into a test tube and mixed with 1.0 ml of reagent (Randox kit). After mixing ,the initial absorbance was read after 30 seconds and again after 1, 2, 3 minutes simultaneously at 340nm using Agilent 8453 spectrophotometer. LDH activity was calculated using the following formula:

U/L = 9683 x DA 340nm/min; Where DA = Change in absorbance.

2.10 DETERMINATION OF SERUM TRIGLYCERIDE LEVEL

Serum triglyceride level was determined according to the method described by Tietz, 1990 (Randox TR 210 kit was used for the quantitative *in vitro* determination of triglyceride in serum).

2.11 DETERMINATION OF SERUM CHOLESTEROL LEVEL

The serum cholesterol was measured according to the method described by Richmond, 1973 (Randox CH 200 kit was used for the quantitative in vitro determination of cholesterol in serum).

2.12 ESTIMATION OF BLOOD GLUCOSE

Finetest Auto-Coding ^{TM} premium blood glucose monitoring system (Glucometer) was used to monitor the blood glucose level. The blood sample was collected via tail bleeding. The blood (1.5 ml sample volume) automatically was drawn onto the test strip channel. After a beeping sound of meter, the test began automatically and result appeared in 9 seconds (Obelis and Sae, 2009).

2.13 BODY WEIGHT MEASUREMENT

The body weights of rats were determined using the weighing balance (Metilado, Suitzerland). The rats were weighed before induction of hypertension, after induction and after treatment and body weights recorded.

2.14 STATISTICAL ANALYSIS

Data were expressed as mean ± SEM. One way analysis of Variance (ANOVA), followed by Benferonis multiple comparison test was applied. A probability value of 0.05 ($P<0.05$) was considered significant.

3. RESULTS AND DISCUSSION

Medicinal herbs have been used as constituents of traditional medicines in Nigeria. Most herbs are relatively inexpensive and easily available and have few adverse effects (Bako et al., 2010). Accordingly, there is growing interest in the use of herbs and their bioactive compounds.

Table 1 presents the mean body weights of rat treated with *Napoleona imperialis* and Propanolol before and 5 days and one week after induction of treatment. There was increase in the body weights of all the animals throughout the experimental period. The increase in body weight was was statistically significant ($P<0.05$). This increase in weight might be due to the extract effect and the nutritive value, the commercial feed given *adlibitum*. This is an indication that the plant is relatively non-toxic as decrease in body weight could mean toxicity.

Napoleona imperialis reduced the pulse rate of the rats after treatment for one week and this was dose dependent as presented in table 2. There was significant ($P<0.05$) increase in the pulse rate of AIHR when compared with the control. The increase in pulse rate of animals or humans could be due to heart diseases or increased metabolic activity (Pagana and Pagana, 2006). Adrenaline increases metabolic activity as well as pulse rate. Treatment with

Napoleona imperialis and Propanolol significantly (P<0.05) decreased the pulse rate of hypertensive rate. This decrease was dose dependant. The reduction in pulse rate of the hypertensive rats confirms the hypotensive effect of *Napoleona imperialis* leaf extract. Its reductive ability is comparative to the standard drug (Propanolol) used.

Table 1. Effect of *Napoleona imperialis* and Propanolol on the body weight (grams) of Rats

Groups	Before induction of hypertension	After induction of hypertension	After treatment of hypertension
Group1: Control rats that received only normal saline	211.28±0.58a	225.75±1.11^{ba}	234.00±1.39^{ca}
Group2: Untreated AIHR	216.75±1.49^{ab}	233.25±0.85^{bb}	242.50±0.75^{cb}
Group3: AIHR treated With NI at 26mg/130±17g b.w.	134.75±2.16ac	143.75±1.93^{bc}	151.00±0.91^{cc}
Group4: AIHR treated with propanolol	126.00±0.91^{ad}	128.50±0.65^{ad}	140.00±0.71^{cd}
Group5: AIHR treated with NI at 52mg/130±17g b.w	113.50±1.55^{ae}	117.00±1.29^{ae}	128.70±1.93^{ce}

Values are expressed as mean ± S.E.M., n = 20.Values along the same column and row with different superscript are considered significant (P< 0.05).
AIHR= Adrenaline induced hypertensive rats, NI= Napoleona imperialis. Group 1 was not induced nor treated and group 2 was induced but not treated.

Pressure over load to the heart, such as from hypertension, results in pathological cardiac hypertrophy (Reddy, 1997; Bishop and Altschuld, 1970). Pathological cardiac hypertrophy induces a reduction of cardiac function (Richey and Brown, 1998) and pathological cardiac hypertrophy result in heart failure (Bishop and Altschuld, 1970; Kagaya et al., 1990). It has been reported that glycolytic energy metabolism is accelerated in hypertensive cardiac hypertrophy induced by pressure over load due to aortic banding or pulmonary hypertension (Iemitsu et al., 2003; Sack and Kelly, 1998).

The heart is known to rely mostly on fat metabolism for energy, but it can also derive energy from several other substances including glucose and lactate (Grynberg and Demaison, 1996). The effect of *Napoleona imperialis* extract and Propanolol on blood glucose, serum triglyceride and cholesterol level of the experimental rats are as presented in table 3.3. There was no significant decrease in blood glucose level of the experimental rats when compared with the control group for both doses of the extract. Significant (P<0.05) decreases in serum triglyceride and cholesterol levels were observed in animals treated with the extract and Propanolol. These significant (P<0.05) decrease in serum triglyceride and cholesterol might be due to the combined effect of the extract, increased metabolic activity of adrenaline used to induce hypertension.

Table 2. Effect of *Napoleona imperialis* and Propanolol on the Pulse Rate of Rats (Beats/min)

Groups	Before induction of hypertension	After induction of hypertension	After treatment of hypertension
Group1: Control rats that received only normal saline	344±0.75^{aa}	343±0.65^{aa}	344±0.04^{xaa}
Group2: Untreated AIHR	339±0.48^{ab}	391±0.91^{ba}	407±1,11^{xcb}
Group3: AIHR treated With NI at 26mg/130±17g b.w.	351±0.75^{ac}	402±1.11^{bc}	359±2.41^{xaai}
Group4: AIHR treated with propanolol	355±0.41^{ad}	411±0.91^{bd}	355±2.06^{xaai}
Group5: AIHR treated with NI at 52mg/130±17g b.w.	358±0.25^{ae}	419±0.71^{be}	349±0.63^{xaaii}

Results are expressed as mean ± S.E.M., n = 20. Values along the same column and row with different superscript are considered significant (P< 0.05).
AIHR= Adrenaline induced hypertensive rats, NI =Napoleona imperialis. Group 1 rats were not induced nor treated and group 2 were induced but not treated.

Table 3. Effect of *Napoleona imperialis* and Propanolol on some Biochemical Parameters (mg/dl)

Groups	Blood Glucose	STL	SCL
Group1: Control rats that received only normal saline	59±1.89^{aa}	66.69±4.51^{ba}	38.26±2.46^{ca}
Group2: Untreated AIHR	53±1.29^{aa}	31.54±2.25^{bb}	26.96±0.52^{cb}
Group3: AIHR treated With NI at 26mg/130±17g b.w	55±2.02aa	45.45±2.68^{bc}	36.94±4.02^{ca}
Group4: AIHR treated with propanolol	48±0.85^{ad}	51.41±3.14^{bc}	40.22±1.49^{ca}
Group5: AIHR treated with NI at 52mg/130±17g b.w	60±2.97^{aa}	47.075±1.40^{bc}	42.09±1.94^{ca}

Results are expressed as mean ± S.E.M., n = 20.Values along the same column with different superscripts is considered significant (P< 0.05).
STL = Serum triglyceride level, SCL = Serum cholesterol level,
AIHR=Adrenaline induced hypertensive rats, NI= Napoleona imperialis.

LDH is found in the cells of almost all body tissues (Stipanuk, 2000). It catalyzes the inter conversion of pyruvate and lactate with concomitant interconversion of NADH and NAD^+ (Groff and Gropper, 2000a). Cellular injury in tissues containing LDH can result in its release into the blood stream. Analysis of the different LDH isoenzyme levels in the blood facilitates the diagnosis of some diseases (Groff and Gropper, 2000b). Iemitsu et al. (2003) reported that the MRNA expression of LDH on the glycolytic metabolic pathway in the heart was markedly higher in spontaneously hypertensive rats compared with controls. LDH release has been associated with cardiac tissue damage. A higher concentration of LDH could be a symptom of heart damage. The plant extract has protective effect on the biological utilization of the lipids than the propanolol.

As presented in table 4, the plant extract reduced the activity of the serum enzymes assayed and the reduction was dose dependent. It was observed that the serum LDH level was significantly lower in *Napoleona imperialis* treated AIHR, suggesting that the extract may protect against cardiac tissue damage. This decrease in activity of the serum enzyme assayed confirmed the hypotensive effect of the extract and the standard drug used.

CPK is associated with hypertension. Hropot et al. (2003) reported that activities of the systosolic enzymes LDH and CPK significantly increases in rats treated with nitric oxide inhibitor, causing a significant increase in systosolic blood pressure. Osbakken et al. (1992) demonstrated diastolic dysfunction in hypertensive dogs using myocardial CPK enzyme kinetics. An epidemiological study showed that systolic blood pressure and diastolic blood pressure levels in healthy black and Asian people in the highest serum CPK tertile were 9 and 5mm Hg higher, respectively, than those in the lowest CPK tertile.

Table 4. Effect of *Napoleona imperialis* Extract and Propanolol on some Serum Enzyme Activity (U/L)

Groups	CPK Activity	LDH Activity
Group1: Control rats that received only normal saline	1729.8 ± 1.48^{aa}	1275.6 ± 7.16^{ba}
Group2: Untreated AIHR	4852.2 ± 1.49^{ab}	3889.9 ± 2.30^{bb}
Group3: AIHR treated With NI at 26mg/130±17g b.w.	2456.1 ± 1.39^{ac}	2303.4 ± 1.00^{bc}
Group4: AIHR treated with propanolol	1639.7 ± 1.73^{aa}	1533.7 ± 8.11^{ba}
Group5: AIHR treated with NI at 52mg/130±17g b.w.	1633.7 ± 1.30^{aa}	1413.5 ± 9.24^{ba}

Results are expressed as mean ± S.E.M, n = 20.Values along the same column with different superscripts are considered significant (P< 0.05).
CPK = Creatine phosphokinase, LDH = Lactate dehydrogenase
AIHR = Adrenaline induced hypertensive rats, NI=Napoleona imperialis.

In addition, high serum CPK activities and high blood pressure level were positively correlated in black people (Richey and Brown, 1998). The results of the present investigation demonstrated that *Napoleona imperialis* is extract and Propanolol significantly ($P<0.05$) decreased CPK and LDH activity, compared with the controls which indicates that the extract may act to reduce blood pressure. The decrease was close dependent.

4. CONCLUSION

In conclusion, our investigation suggests that *Napoleona imperialis* leaf extract has got profound hypotensive activity and this study has correlation with previously reported investigations using other plants. The mechanism by which *Napoleona imperialis* lowers blood pressure is not yet fully established. However, the hypertensive effect may be due to the stimulation of muscarinic receptors of the parasympathetic nerve by the compounds or their actions as an antagonist of α_2 – adrenergic receptors but it may act as Ca^+ ion channel block (Amran et al., 2004). The intake of *Napoleona imperialis* as medicine or as supplement in diet might have potential benefit in the treatment of hypertension. In this respect, herbal drugs are helpful and render encouraging results in comparison to synthetic drugs due to their fewer side effects and easy availability.

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