Title page

Effect of methanolic extract of *Ocimum gratissimum* on blood pressure, some electrolytes, renal and cardiac biomarkers in 8% NaCl induced hypertensive male wistar rats.

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Abstract

Background*: Ocimum gratissimum* (OG) is a shrub belonging to the family of Lamiaceae. It is commonly called scent leaf or clove basil and it is found in many tropical countries. Studies have shown that the leaf extract of *Ocimum gratissimum* possess medicinal properties.
Aim: The effect of methanolic extract of *Ocimum gratissimum* on blood pressure, electrolytes, renal and cardiac biomarkers in 8% NaCl- induced hypertensive male Wistar rats.
Methodology: Forty Wistar rats (120-160)g were assigned to 5 groups of eight rats each. Group 1, 2, 3, 4 and 5 constitute the normal, hypertensive group, OG (200 mg/kg bwt) group, OG (400 mg/kg bwt) group and reference drugs (lisinopril, 30 mg/kg) group respectively. Group 3, 4 and 5 were given the extract and reference drug through oral gavage. All groups except group 1 were induced with 8% NaCl from 0-4weeks before treatment with OG and reference drug from 5-8 weeks. Electrolytes and other biochemical parameters were assayed using standard methods.
Results: The phytochemical results revealed the presence of phenol, flavonoids, alkaloids, phytate, tannis and saponin. At 4 weeks (after induction), systolic blood pressure (SBP), diastolic blood pressure (DBP), serum sodium, chloride, urea, and creatinine significantly ($p<0.05$) increased while serum potassium significantly ($p<0.05$) decreased in all the groups except group 1. At 8 weeks, after treatment with OG (200 mg/kgbwt), OG (400 mg/kgbwt) and lisinopril (30mg/kg), SBP, DBP, serum sodium, chloride, urea, and creatinine significantly($p<0.05$) decreased while serum potassium significantly ($p<0.05$) increased. Creatine kinase (CK) and CK-MB however, were not significantly altered after the 4th and 8th week.
Conclusion: OG extract possesses an antihypertensive effect and enhances the proper functioning of the kidney. It may also be useful in hypertensive condition due to its nephroprotective effect at 200mg/kgbwt and 400 mg/kgbwt.
Key words: *Ocimum gratissimum* (OG), phytochemical analysis, blood pressure, electrolytes, renal markers, cardiac markers, NaCl hypertension.

1.0 INTRODUCTION

Ocimum gratissimum (OG) is an edible plant belonging to the family of Lamiaceae. It is commonly called scent leaf or clove basil and it is found in many tropical countries. The genus, *Ocimum* (Lamiaceae), comprises 65 aromatic species, distributed in tropical and subtropical regions of the world including Africa and Asia [1]. Various *Ocimum* species, including *Ocimum basilicum* and *Ocimum gratissimum*, have important culinary and pharmacological uses. Their culinary and pharmacological values are attributed mainly to their aromatic compounds [2]. Hence, the leaves are used as a fragrance and flavoring agent in a variety of products including food, beverages, condiments, and oral care products [3]. *Ocimum gratissimum* leaves extract possesses anti-arthritic [4], hypoglycemic [1], anticonvulsant and anxiolytic activities [5]. The leaf extract of *Ocimum gratissimum* showed anti-diabetic properties [6, 7] and antibacterial activities[8, 9]. It has mosquito repellent and mosquitocidal potential [10], as well as hepatoprotective effect [11, 12]. Hypertension is a serious public health problem due to increasing incidence and prevalence. It is a significant risk factor for heart disease, stroke and other cardiovascular diseases [13] Although antihypertensive drug are used in the management of hypertension, some of these drugs are faced with the problem of fake and adulterated drugs, drug abuse, high cost of drugs and drug side effects such as erectile dysfunction, extra urination,

weakness and asthma symptoms. There is paucity of literature on the use of this plant as antihypertensive. Also, natural plant products are increasingly investigated for their therapeutic potentials. Hence, the need to look at our indigenous plant *Ocimum gratissimum* which are natural, safe, readily available at almost no cost when compared with conventional drugs for their possible nutraceutical benefit and therapeutic properties that may aid in the prevention and management of hypertension when taken as a supplement.

The aim of the study is to determine the antihypertensive effect of *Ocimum gratissimum* that may possibly be used in the management and prevention of hypertension.

1) To determine the phytochemical and mineral contents of methanolic leaf extract of *Ocimum gratissimum(OG).*
2) To determine the effect of OG on blood pressure, electrolytes, urea, creatinine, Creatine kinase (CK) and Creatine kinase-MB in 8% NaCl induced hypertensive male Wistar rats in order to ascertain its antihypertensive effect when compared with the reference drug (lisinopril).

2.0 MATERIALS AND METHODS

2.1 Plant extraction (maceration method) [14].

Fresh leaves of *Ocimum gratissimum* (scent leaf) were air-dried at room temperature. Air-dried leaves of the plant were milled into powder. The powdered leaves were weighed and macerated into methanol (500g of the plant material to 2.5liters of methanol in a stopper) for 5 days with occasional shaking to soften and break the plant cell wall to ensure sufficient extraction of the active photochemical. At the end of five days, the methanolic extract was filtered using whatman No.1 filter paper and the filtrate concentrated to dryness under reduced pressure at 60$\pm$ 1^{o} C in a rotary evaporator at 45^{o}C, weighed and stored frozen until used. The exact weight of dried extract from 500g powder was 87.8g and gave a percentage yield of 17.56%. The extract was dissolved in tween80 and was given to the animals at graded doses of 200mg/kgbwt and 400mg/kgbwt.

2.2 Procurement and care of animals

Forty male Wistar rats weighing 120-160g were obtained from university of Nigeria Nsukka and housed in cages in the animal facility. They were fed with clean water and rat chow *ad libitum*. The rats were allowed to acclimatize for 2 weeks during which the rat local restrainer was included in their cages to prepare the rat for blood pressure measurement. The rats were maintained under good laboratory conditions at a temperature of 22±2^{o}C, relative humidity of 50± 5% and photoperiod of 12hr (12h-dark and 12h-light cycle).

2.3 Exprimental Design

The animal study protocol was designed into two stages. The first stage is the induction period while the second stage is the treatment period.

The animals were grouped into five different groups with each group assigned different numbers (1 – 5). Each group (Group 1, 2, 3, 4 and 5) contains 8 rats and were fed with normal rat chow and clean water *ad libitum* for the period of the study.

Group 1 (negative control) - Normal rat chow and water *ad libitum*

Group 2 (Untreated hypertensive) -Induction with 8% NaCl for 4weeks and subsequently no treatment

Group 3(200mg/kgbwt OG) -Induction with 8% NaCl for 4weeks and subsequent treatment with 200mg/kgbwt OG for another 4 weeks.

Group 4(400mg/kgbwt OG) -Induction with 8% NaCl for 4weeks and subsequent treatment with 400mg/kgbwt OG for another 4 weeks

Group 5(30mg/kg Lisinopril) -Induction with 8% NaCl for 4weeks and subsequent treatment with lisinopril for another 4 weeks.

2.4 Blood pressure measurement in rats using Kent Scientific CODA machine

Volume pressure recording (VPR) method of blood pressure measurement was used as suggested by the American Heart Association[15]. Blood pressure was measured using VPR non-invasive blood pressure monitoring system (CODA-6) Kent Scientific, Torrington, CT).

Blood pressure measurement procedure

The method of measurement of VPR is based on tail volume. At the start of the measurement cycle, blood is pushed from the tail by the VPR cuff and then the occlusion cuff inflates to prevent blood flow back into the tail. When the occlusion cuff deflates, blood begins to flow back into the tail, increasing the tail volume. The occlusion cuff pressure at which the tail volume increases is the SBP. The tail volume will continue to increase as the occlusion cuff deflates until blood flow into and out of the tail equalizes; the occlusion cuff pressure at this point is the DBP.

2.5 LD 50: Acute toxicity studies of methanolic extract of *Ocimum gratissimum*(MEOG)

The acute toxicity of MEOG was determined by Lorke's method[16] as described by Enegide[17]. Rats were divided into two phases. In the first phase of the study, 9 rats were divided into 3 groups of 3 rats each and they were treated with MEOG through oral gavage at the doses of 10, 100 and 1000 mg/kg respectively. The rats were observed for behavioural change and lethality in the first 24 hrs.

In the second stage, 3 rats were divided into 3 groups of 1 rat each and they were treated with MEOG through oral gavage at the doses of 1600, 2900 and 5000mg/kg. The general behaviour of the animals were observed continuously for 1 hr after treatment and then intermittently for 4 hrs, then hourly for the next 24hrs. The LD_{50} was determined using the formular below

$$LD_{50} = \sqrt{a \times b}$$

Where a = minimal lethal dose

b = maximal survival dose

$OG\ LD_{50} = \sqrt{a \times b} = \sqrt{1600 \times 5000} => \sqrt{8000000} = 2828$

2.6 Biochemical methods/assay

Determination of Alkaloids

Alkaloids were determined using the method of Harborne [18] .

Procedure:

Five grams (5g) of the sample was weighed into a 250ml beaker and 200ml of 20% acetic acid in ethanol was added and covered and allowed to stand for 4 hours at 25°c. This was filtered with filter paper No. 42 and the filtrate was concentrated using a water bath (Memmert) to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitate was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute NH_4OH (1% ammonia solution). Then, filter with pre-weighed filter paper. The residue on the filter paper is the alkaloid, which is dried in the oven (precision electrothermal model BNP 9052 England) at 80°c. The alkaloid content was calculated and expressed as a percentage of the weight of the sample analyzed.

Calculation:

$$\%\text{weight of alkaloid} = \frac{\text{weight of filter paper with residue - weight of filter paper}}{\text{Weight of sample analyzed}} \times 100$$

Determination of Flavonoids

Flavonoids were determined using the method of Bohm and Kocipai-Abyazan [19].

Procedure:

10g of the plant sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No. 42 (125mm).

The filtrate was later transferred into a crucible and evaporated into dryness over a waterbath and weighed to a constant weight.

Calculation:

$$\%\text{flavonoids} = \frac{(\text{weight of crucible + residue}) - (\text{weight of crucible})}{\text{Weight of sample analyzed}} \times 100$$

Determination of Saponin

Saponin was determined using the method of Obadoni and Ochuko[20].

Procedure:

20 g of each grounded sample was put into a conical flask and 100 cm^3 of 20% aqueous ethanol was added. Then the flask was heated on a hot water bath for 4 hrs with constant stirring at about 55°C. The mixture was then filtered and the residue was again extracted with another 200 ml 20% ethanol. The combined extract was reduced to 40 ml on a hot water bath at about 90°C. The concentrate was transferred into a 250 ml separatory funnel, added 20 ml diethyl ether in it followed by vigorous shaking. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in oven, weighed and saponin content was calculated as percentage

Calculation:

$$\%\ \text{saponin content} = \frac{(\text{weight of filter paper + residue}) - (\text{weight of filter paper})}{} \times 100$$

Weight of sample analyzed

Determination of Tannin

Tannin content of the sample was determined by Follins Dennis titration method as described by Pearson [21].

Procedure:

The follins Dennis titrating method as described by Pearson (1974) was used. To 20g of the crushed sample in a conical flask was added 100mls of petroleum ether and covered for 24 hours. The sample was then filtered and allowed to stand for 15 minutes allowing petroleum ether to evaporate. It was then re-extracted by soaking in 100ml of 10% acetic acid in ethanol for 4hrs. The sample was then filtered and the filterate collected. Then 25ml of NH_4OH were added to the filterate to precipitate the alkaloids. The alkaloids were heated with electric hot plate to remove some of the NH_4OH still in solution. The remaining volume was measured to be 33ml. 5ml of this was taken and 20ml of ethanol was added to it. It was titrated with 0.1 M NaOH using phenolphthalein indicator until a pink end point is reached.

Calculation:

Tannin content was then calculated in % ($C_1V_1 = C_2V_2$) molarity.

Where:

C_1 = conc. of Tannic Acid

C_2 = conc. Of Base

V_1 = Volume of Tannic acid

V_2= Volume of Base

Therefore $C_1 = \frac{C_2 V_2}{V_1}$

% of tannic acid content = $\underline{C_1 \times 100}$

Weight of sample analyzed

Determination of Total Phenols

Total phenols were determined by spectrophotometric method as described by Khan *et al*, [22].

Procedure:

Two grams (2 g) of each plant sample was defatted with the help of 100 ml of diethyl ether using a soxhlet apparatus for 2 h. The fat free sample was boiled with 50 ml of ether for 15 min for the extraction of phenolic component. 5 ml of the extract was pipetted into a 50 ml flask and 10 ml distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amylalcohol were also added in it. The samples were made up to mark and left to react for 30 min. Colour was developed and its absorbance was measured at 505 nm.

Determination of Phytate Contents

Phytate contents were determined using the method of Lucas and Markakes [23].

Procedure:

0.2g of each samples was weighed into different 250ml conical flasks. Each sample was soaked in 100 ml of 2% concentrated HCI for 3 hours.The samples were then filtered. 50 ml of each filtrate was placed in 250ml beaker and 100ml distilled water added to each sample. 10 ml of 0.3% ammonium thiocynate solution was added as indicator and titrated with standard iron (III) chloride solution which contained 0.00195g iron per l ml.

Calculation:

$$\text{Phytic acid} = \frac{\text{Titre value} \times 0.00195 \times 1.19 \times 100}{\text{Wt of sample}}$$

Determination of mineral content of *Ocimum gratissimum*

Mineral content was determined by Association of Official Analytical Chemists methods[24] using the flame system of the atomic absorption spectrophotometry (AAS), (Varian Spectr AA240, USA).

Procedure:

Ocimum gratissimum was ashed at 550°C overnight and the ash was dissolved in concentrated nitric acid and filtered, diluted to 50 ml with deionized water and the absorbance of the samples was read directly on the AAS. Working standard solutions of potassium, calcium, magnesium, phosphorus and sodium were prepared from stock standard solution (1000 ppm), in 2 N $HN0_3$ and absorbance was noted for standard solution of each element and samples using atomic absorption spectrophotometer (AAS). Calibration curve for each metal was prepared by plotting the absorbance of standards versus their concentrations. A blank reading was also taken and necessary corrections were made during the calculation of concentration of various elements.

Determination of Urea Level

Blood urea was determined by the method described by Kassirer [25].

Urea is decomposed by urease to form ammonia and carbon dioxide. Ammonia combines with 2-oxo-glutarate in presence of glutamate dehydrogenase and NADH to form L-Glutamate and NAD. The rate of NAD formation measured at 340 nm is directly proportional to the amount of blood urea.

Procedure:

Test tubes were labelled: reagent blank, Standard, Control and Sample. 1.0 ml of enzyme reagent (urease, ADP), followed by 0.01 ml of sample/standard/control in their respective tubes and mixed thoroughly. These solutions were allowed to stand at 37°C for one minute. 0.250 ml of 2-

oxoglutarate and NADH reagent was added to all the tubes and mixed. The initial absorbance was measured after 30 seconds (AI) and the second reading taken at exactly one minute (A2) measured against the reagent blank at 340 nm.

Calculation:

The mean change in absorbance readings were calculated thus (ΔA2-Al/min)

$$\text{Urea (mmol/l)} = \frac{\Delta A_{sample}}{\Delta A_{std}} \times \text{Concentration of standard}$$

3.5.13 Determination of Creatinine Level

Serum creatinine was estimated by Jaffe's method as described by Laron[26].

Serum creatinine in alkaline medium reacts with Picric acid to produce orange colour that absorbs light at 492 nm. The rate of increasing absorption is directly proportional to the amount of creatinine in the sample.

creatinine + Sodium Picrate $\xrightarrow{\text{Alkaline pH}}$ Creatinine-Picrate complex (yellow-orange)

Procedure:

Test tubes were labelled: Blank, Standard, Control, and Sample. One (1.0) ml of reagent was added into a test tube and allowed to equilibrate at 37°C. The spectrophotometer was zeroed with the reagent blank at 510 nm. 0.05 ml of sample or standard or control was added to the working reagent, mixed and incubated at 37°C. The first reading was taken after 30 seconds and the absorbance recorded as A1. At exactly sixty seconds after the A1 reading, the solution was read again and the absorbance recorded as A2. The change in absorbance (ΔA/min) was calculated by subtracting (A2 - AI).

Calculation:

$$\text{Creatinine } (\mu mol/l) = \frac{\Delta Abs.\ of\ unknown}{\Delta Abs.of standard} \times \text{Concentration of standard}$$

Determination of CK Activity

CK activity was assayed using the methods of Szasz [27] as recommended by the IFCC.

Creatine phosphate + ADP $\xrightarrow{\text{creatine kinase}}$ creatine + ATP

ATP + glucose $\xrightarrow{\text{hexokinase}}$ glucose-6-phosphate + ADP

Glucose-6-phosphate + $NADP^+$ $\xrightarrow{\text{G6PDH}}$ 6-phosphogluconate + NADPH + H^+

The rate of NADPH formation is directly proportional to the catalytic CK activity. It is determined by measuring the increase in absorbance at 340 nm.

Creatine kinase-MB determination

Immunoinhibition method was used in estimation of CK-MB [28]. The sample was incubated in the CK-MB reagent which includes the CK-M antibody. The activity of the non-inhibited CK-B was then determined using the following series of reaction. CK-B catalyses the reversible phosphorylation of ADP, in the presence of creatine phosphate to form ATP and creatinine. The auxillary enzyme hexokinase (HK) catalyses the phosphorylation of glucose by ATP format, to produce ADP and glucose-6-phosphate is oxidized to 6-phosphogluconate with concomitant production of NADH. The rate of NADH formation, measured at 340nm, is directly proportional to serum CK-B activity.

2.7 Statistical Analysis

The version 23 of Statistical Package for Social Sciences (SPSS) was used in statistical analysis. The variables were expressed as mean±SD. The independent student t-test was used to assess significant mean difference between two independent groups, while paired t-test was used to assess the mean difference between two related groups. Analysis of Variance (ANOVA) was also used and POST HOC was used to determine the significant difference within the groups. The level of significance was considered at $P<0.05$.

3.0 RESULTS

Phytochemical result revealed the presence of phenol, steroid, alkaloids, flavonoids, saponin, tannis and phytate while the mineral content revealed the presence of potassium, phosphorus, calcium, magnesium and sodium (table 1).

Systolic blood pressure, diastolic blood pressure, serum sodium, chloride, urea and creatinine were significantly increased ($p<0.05$) while serum potassium and bicarbonate were significantly decreased ($p<0.05$) after 4 weeks induction of hypertension. After administration of methanolic extract of *Vernonia amgydalina* and *Ocimum gratissimum* at 200 mg/kgbwt and 400mg/kgbwt systolic blood pressure, diastolic blood pressure, serum sodium, chloride, urea and creatinine were significantly decreased ($p<0.05$) while serum potassium significantly increased ($p<0.05$) after 4 weeks induction of hypertension in a dose-dependent manner(table 2-9). Serum creatine and creatine-kinase did not differ significantly at 4 weeks and 8 weeks respectively (table 10-11).

4.0 DISCUSSION

Hypertension was induced in male Wistar rats to look at the possible prevention and management of hypertension using methanolic extract of *Ocimum gratissimum(OG)* in comparison with a reference

drug, lisinopril. Induction of hypertension was achieved through oral gavage administration of 8% NaCl in water for a period of 4 weeks. The induction process was in agreement with work done by Rini [29]. The oral dosing of 8% NaCl was performed without anaesthesia. The NaCl induced hypertension may have been achieved through increased sympathetic nerve activity, a major trigger of vasoconstriction as well as the activation of renin angiotension aldosterone system[30]. Phytochemical analysis revealed the presence of steroid, flavonoids, phenol, alkaloids, saponin, tannis and phytate while mineral content revealed the presence of potassium, magnesium, phosphorus, calcium and sodium. The phytochemical result obtained was in accordance with Udochukwu[31]. After 4 weeks, systolic and diastolic blood pressures were significantly elevated in all the groups except group 1 which is the control group. On treatment with 200mg/kg OG, 400mg/kgOG and 30mg/kg lisinopril, the elevated SBP and DBP significantly reduced ($P<0.05$). The significant reduction in SBP and DBP may be related to the high concentration of potassium and magnesium in methanolic extract of *Ocimum gratissimum*. Potassium and magnesium are important for muscle function, which includes relaxing the walls of blood vessels. Normal potassium levels are important for the conduction of electrical signals in the nervous system and in the heart. This protects against an irregular heartbeat. Potassium counters the effect of sodium which was significantly increased after 4 weeks induction with NaCl. At 8 weeks, serum sodium level was significantly reduced and potassium was significantly increased. Urea and creatinine were significantly increased at 4weeks suggesting impairment of renal function or loss of structural integrity of the kidney cell membrane but at 8weeks, the elevated renal parameters were reduced significantly. The observed changes is in accordance with the report of Ogundipe [32] who reported on the effects of two weeks administration of *Ocimum gratissimum* leaf on feeding pattern and markers of renal function in rats treated with gentamicin. Ogundipe observed a decrease in urea and creatinine although it was for a shorter period of 2 weeks. Reduction in the renal markers suggests that methanolic extract of *Ocimum gratissimum* supplementation may lead to increase in glomerular filtration rate hence maybe helpful in hypertensive nephropathy. Methanolic extract of *Ocimum gratissimum* did not significantly affect the serum levels of creatine kinase(CK)and creatine kinase-MB which makes the cardioprotective effect of the extract uncertain in this study.

5.0 CONCLUSION

Ocimum gratissimum possesses antihypertensive effect and enhances the proper functioning of the kidney. It may also be useful in hypertensive condition due to its nephroprotective effect at 200mg/kgbwt and 400 mg/kgbwt.

6.0 ACKNOWLEDGEMENT

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7.0 COMPETING INTEREST

Authors have declared that no competing interests exist.

8.0 AUTHOR'S CONTRIBUTION

Onyema-iloh OB- wrote the protocol and carried out sample analysis

Iloh EO- carried out phytochemical and mineral analysis

Meludu SC, Dioka CE- assisted in study design and protocol

Usman OS- managed statistical analysis

Onyegbule OA, Olugbenga EB-managed the literature searches

9.0 Ethical Approval

The ethical approval for this study was obtained from the Ethics Committee of Nnamdi Azikiwe University Teaching Hospital, Nnewi with approval number: NAUTH/CS/66/VOL.9/145/2016/119.

Consent: NA

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11.0 TABLES

Table 1: Phytochemical and mineral component of methanolic extract of *Ocimum gratissimum*(OG)

PHYTOCHEMICAL(mg/100g)	***Ocimum gratissimum(OG)***
Phenol	0.52
Steroid	0.24
Alkaloids	1.04
Flavonoids	3.02
Saponins	3.35
Tannis	2.16
Phytate	4.24
MINERAL COMPONENTS(ppm)	
Potassium	42.60
Calcium	32.60
Magnesium	60.20
Phosphorus	12.35
Sodium	15.80

Table 2. Effect of methanolic extract of *Ocimum gratissimum* (OG) on systolic blood pressure (SBP) at 0week (baseline), 4 weeks (after induction of hypertension) and 8weeks (after treatment with OG)

SBP (n=8)	**0 week**	**4 weeks**	**8 weeks**
Neg Control	81.00±11.23	82.66±23.71	81.00±11.21
Untreated group	83.50±10.32	162.33±6.12 [a]	190.17±5.49 [b,*]
OG 200mg/kg bwt	84.83±11.35	178.16±13.15 [a]	123.83±3.48 [b,c,*]
OG 400mg/kg bwt	82.66±11.20	174.17±13.39 [a]	92.66±6.94 [b,c,,*]
Lisinopril 30mg/kg bwt	82.23±19.66	168.66±6.88 [a]	84.66±4.63 [b]

Values expressed as mean± S.D. **a=**Statistically significant at P<0.05 when compared between 0 week and 4 weeks, b= when compared between 4 weeks and 8 weeks and c= when compared between 0 week and 8 weeks and * when compared within the groups and control

Table 3. Effect of methanolic extract of *Ocimum gratissimum* (OG) on Diastolic blood pressure (DBP) at 0week (baseline), 4 weeks (after induction of hypertension) and 8weeks (after treatment with OG)

DBP (n=8)	**0 week**	**4 weeks**	**8 weeks**

Neg Control	60.66±11.91	60.67±11.91	60.67±11.91
Untreated group	61.50±8.57	124.66±16.35[a]	132.83±10.92[b,c,*]
OG 200mg/kg bwt	61.16±8.70	127.66±13.4[a]	74.00±8.62[b,*]
OG 400mg/kg bwt	63.83±5.60	126.67±13.78[a]	63.00±8.83[b]
Lisinopril 30mg/kg bwt	63.80±9.57	126.66±13.41[a]	74.66±4.63[b,*]

Values expressed as mean± S.D. **a=**Statistically significant at P<0.05 when compared between 0 week and 4 weeks, b= when compared between 4 weeks and 8 weeks and c= when compared between 0 week and 8 weeks and * when compared within the groups and control

Table 4. Effect of methanolic extract of *Ocimum gratissimum* (OG) on serum sodium (Na) level at 0week (baseline), 4 weeks (after induction of hypertension) and 8weeks (after treatment with OG)

Na (n=8)	**0 week**	**4 weeks**	**8 weeks**
Neg Control	136.67±1.36	136.62±1.32	136.67±1.36
Untreated group	138.16±1.94	168.50±3.45[a]	174.83±6.49[b,c,*]
OG 200mg/kg bwt	136.67±1.36	182.33±4.68[a]	155.67±8.56[b,c,*]
OG 400mg/kg bwt	138.17±1.94	180.83±17.51[a]	143.33±8.21[b,c,*]
Lisinopril 30mg/kg bwt	138.67±1.50	181.10±7.51[a]	149.50±7.28[b,c,*]

Values expressed as mean± S.D. **a=**Statistically significant at P<0.05 when compared between 0 week and 4 weeks, b= when compared between 4 weeks and 8 weeks and c= when compared between 0 week and 8 weeks and * when compared within the groups and control.

Table 5. Effect of methanolic extract of *Ocimum gratissimum* (OG) on serum Potassium (K) level at 0week (baseline), 4 weeks (after induction of hypertension) and 8weeks (after treatment with OG)

K (n=8)	**0 week**	**4weeks**	**8 weeks**
Neg control	5.53±0.16	5.50±0.12	5.53±0.16
Untreated group	5.53±0.16	3.40±0.36[a]	3.20±0.32[c,*]
OG 200mg/kg bwt	5.43±0.07	4.98±0.63[a]	5.78±0.29[b]
OG 400mg/kg bwt	5.45±0.09	5.00±0.95[a]	5.72±0.34[b]
Lisinopril 30mg/kg bwt	5.47±0.08	4.83±0.84[a]	4.82±0.84[c,*]

Values expressed as mean± S.D. **a=**statistically significant at P<0.05 when compared between 0 week and 4 weeks, b= when compared between 4 weeks and 8 weeks and c= when compared between 0 week and 8 weeks and * when compared within the groups and control.

Table 6. Effect of methanolic extract of *Ocimum gratissimum* (OG) on serum chloride (Cl) level at 0week (baseline), 4 weeks (after induction of hypertension) and 8weeks (after treatment with OG)

Cl(mmol) n=8	**0 week**	**4 weeks**	**8 weeks**
Neg control	105.33 ± 2.41	105.31 ± 2.42	105.32 ±2.42
Untreated group	105.83 ± 0.75	125.00 ± 9.65[a]	130.33 ±3.50 [b,c,*]
OG 200mg/kg bwt	105.83 ± 1.17	121.33 ± 3.72 [a]	104.83 ±3.76 [b]
OG 400mg/kg bwt	105.33 ± 2.42	123.00 ± 4.24 [a]	105.50 ±3.94[b]
Lisinopril 30mg/kg bwt	103.67 ± 2.58	128.00 ± 3.63 [a]	128.00 ± 3.63[c,*]

Values expressed as mean± S.D. **a=**Statistically significant at P<0.05 when compared between 0 week and 4 weeks, b= when compared between 4 weeks and 8 weeks and c= when compared between 0 week and 8 weeks and * when compared within the groups and control.

Table 7. Effect of methanolic extract of *Ocimum gratissimum* (OG) on serum bicarbonate (HCO_3) level at 0week (baseline), 4 weeks (after induction of hypertension) and 8weeks (after treatment with OG)

HCO_3 (mmol/l) n=8	**0week**	**4weeks**	**8 weeks**
Neg control (G1)	25.16 ± 1.72	25.13 ± 1.82	25.17 ± 1.78
Untreated group (G2)	26.50 ± 1.64	24.50 ± 0.54[a]	23.17 ± 1.69[c]
OG 200mg/kg bwt(G5)	25.50 ± 1.64	17.17 ± 2.23 [a]	17.17 ± 2.24[c,*]
OG 400mg/kg bwt(G6)	25.16 ±1.72	17.16 ± 3.31 [a]	16. 63 ± 2.73 [c,*]
Lisinopril 30 mg/kg bwt(G7)	26.00 ± 1.41	20.33 ± 1.86 [a]	20.33 ± 1.86[c,*]

Values expressed as mean± S.D. **a=**Statistically significant at P<0.05 when compared between 0 week and 4 weeks, b= when compared between 4 weeks and 8 weeks and c= when compared between 0 week and 8 weeks and * when compared within the groups and control.

Table 8. Effect of methanolic extract of *Ocimum gratissimum* (OG) on serum urea level at 0week (baseline), 4 weeks (after induction of hypertension) and 8weeks (after treatment with OG)

Urea(mmol/l) n=8	**0 week**	**4 weeks**	**8 weeks**
Neg control (G1)	5.63 ± 0.03	5.61 ± 0.01	5.62 ± 0.02
Untreated group (G2)	5.62 ± 0.02	7.33 ± 0.67[a]	7.82 ± 0.46 [c,*]
OG 200mg/kg bwt(G5)	5.62 ± 0.01	6.86 ± 0.52 [a]	5.78 ± 0.48 [b]
OG 400mg/kg bwt(G6)	5.63 ± 0.01	7.11 ± 0.41 [a]	5.68 ± 0.33 [b]

Lisinopril 30mg/kg bwt(G7)	5.63 ± 0.01	6.85 ± 0.57 [a]	6.85 ± 0.56 [c,*]

Values expressed as mean± S.D. **a=**Statistically significant at P<0.05 when compared between 0 week and 4 weeks, b= when compared between 4 weeks and 8 weeks and c= when compared between 0 week and 8 weeks and * when compared within the groups and control.

Table 9. Effect of methanolic extract of *Ocimum gratissimum* (OG) on serum creatinine level at 0week (baseline), 4 weeks (after induction of hypertension) and 8weeks (after treatment with OG)

Creatinine(µmol/l)n=8	**0 week**	**4 weeks**	**8 weeks**
Neg control	47.18 ± 1.49	47.17 ± 1.47	47.16 ±1.47
Untreated group	47.67± 1.21	63.35 ± 3.77 [a]	61.17 ± 4.95 [b,*]
OG 200mg/kg bwt	47.16 ± 1.47	62.00 ± 5.32 [a]	51.33 ± 5.71 [b,c,*]
OG 400mg/kg bwt	47.33 ± 1.21	63.33 ± 1.03 [a]	49.50 ± 3.72 [b]
Lisinopril 30mg/kg bwt	47.00 ± 0.89	66.00 ± 2.75 [a]	68.50 ± 13.40 [b,c,*]

Values expressed as mean± S.D. **a=**Statistically significant at P<0.05 when compared between 0 week and 4 weeks, b= when compared between 4 weeks and 8 weeks and c= when compared between 0 week and 8 weeks and * when compared within the groups and control.

Table 10. Effect of methanolic extract of *Ocimum gratissimum* (OG) on serum creatine kinase(CK) level at 0week (baseline), 4 weeks (after induction of hypertension) and 8weeks (after treatment with OG)

CK(U/L) n=8	**0 week**	**4 weeks**	**8 weeks**
Neg control	45.36±0.09	44.63±1.27	44.49±1.83
Untreated group	45.10±0.17	44.95±1.75	46.99±1.54
OG 200mg/kg bwt	45.37±0.07	44.39±0.92	43.58±1.21
OG 400mg/kg bwt	45.13±0.24	43.92±0.95	43.72±0.66
Lisinopril 30mg/kg bwt	44.97±0.94	43.82±1.08	43.48±1.36

Values expressed as mean± S.D. **a=**Statistically significant at P<0.05 when compared between 0 week and 4 weeks, b= when compared between 4 weeks and 8 weeks and c= when compared between 0 week and 8 weeks and * when compared within the groups and control.

Table 11. Effect of methanolic extract of *Ocimum gratissimum* (OG) on serum CK-MB level at 0week (baseline), 4 weeks (after induction of hypertension) and 8weeks (after treatment with OG)

CK-MB(U/L) n=8	**0 week**	**4 weeks**	**8 weeks**
control	14.47±0.03	14.48±0.03	14.48±0.03
Untreated group	14.48±0.03	15.28±0.05	15.15±1.00
OG 200mg/kg bwt	14.47±0.03	15.14±0.39	14.33±0.03
OG 400mg/kg bwt	14.49±0.01	15.19±0.14	14.32±0.08
Lisinopril 30mg/kg bwt	14.48±0.02	15.39±0.08	15.18±0.18

Values expressed as mean± S.D. **a=**Statistically significant at P<0.05 when compared between 0

week and 4 weeks, b= when compared between 4 weeks and 8 weeks and c= when compared between 0 week and 8 weeks and * when compared within the groups and control.