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

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* possessed 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 drug (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 for 0-4weeks before treatment with OG and reference drug for 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 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

33 *Ocimum gratissimum* (OG) is an edible plant belonging to the family of Lamiaceae. It is
34 commonly called Scent leaf or clove basil and it is found in many tropical countries. The leaf
35 extract of *Ocimum gratissimum* showed anti-diabetic properties^{1,2} and antibacterial activities^{3,4}.
36 It has mosquito repellent and mosquitocidal potential⁵, as well as hepatoprotective effect^{6,7}.
37 Hypertension is a serious public health problem due to increasing incidence and prevalence. It is
38 a significant risk factor for heart disease, stroke and other cardiovascular diseases⁸ Although
39 antihypertensive drug are used in the management of hypertension, some of these drugs are faced
40 with the problem of fake and adulterated drugs, drug abuse, high cost of drugs and drug side
41 effects such as erectile dysfunction, extra urination, weakness and asthma symptoms. There is
42 paucity of literature on the use of this plant as antihypertensive. Also, natural plant products are
43 increasingly investigated for their therapeutic potentials. Hence, the need to look at our
44 indigenous plant *Ocimum gratissimum* used as vegetables because they are natural, safe, readily
45 available at almost no cost when compared with conventional drugs for their possible therapeutic
46 properties that may aid in the prevention and management of hypertension when taken as a
47 supplement.

48 **1.1 Aim :** The aim of the study is to determine the antihypertensive effect of *Ocimum*
49 *gratissimum* that may possibly be used in the management and prevention of hypertension.

50 **1.2 Objectives of the study:**

511) To determine the phytochemical and mineral contents of methanolic leaf extract of *Ocimum*
52 *gratissimum*(OG).

532) To determine the effect of OG on blood pressure, electrolytes, urea, creatinine, Creatine kinase
54 (CK) and Creatine kinase-MB in 8% NaCl induced hypertensive male wistar rats in order to
55 ascertain its antihypertensive effect when compared with the reference drug (lisinopril).

56 **2.0 MATERIALS AND METHODS**

57 **2.1 Plant extraction (maceration method)⁹**

58 Fresh leaves of *Ocimum gratissimum* (scent leaf) were air-dried at room temperature. Air-dried
59 leaves of the plant were milled into powder. The powdered leaves were weighed and macerated
60 into methanol (500g of the plant material to 2.5liters of methanol in a stopper) for 5 days with

61 occasional shaking to soften and break the plant cell wall to ensure sufficient extraction of the
62 active photochemical. At the end of five days, the methanolic extract was filtered using whatman
63 No.1 filter paper and the filtrate concentrated to dryness under reduced pressure at $60 \pm 1^{\circ} \text{C}$ in a
64 rotary evaporator at 45°C , weighed and stored frozen until used. The exact weight of dried
65 extract from 500g powder was 87.8g and gave a percentage yield of 17.56%. The extract was
66 dissolved in tween80 and was given to the animals at graded doses of 200mg/kgbw and
67 400mg/kgbw.

68 **2.2 Procurement and care of animals**

69 Forty male wistar rats weighing 120-160g were obtained from university of Nigeria Nsukka and
70 housed in cages in the animal facility. They were fed with clean water and rat chow *ad libitum*.
71 The rats were allowed to acclimatize for 2weeks during which the rat local restrainer was
72 included in their cages to prepare the rat for blood pressure measurement. The rats were
73 maintained under good laboratory conditions at a temperature of $22 \pm 2^{\circ} \text{C}$, relative humidity of
74 $50 \pm 5\%$ and photoperiod of 12hr (12h-dark and 12h-light cycle).

75 **2.3 Animal Study Design**

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

78 The animals were grouped into five different groups with each group assigned different numbers
79 (1 – 5). Each group (Group 1, 2, 3, 4 and 5) contains 8 rats and were fed with normal rat chow
80 and clean water *ad libitum* for the period of the study.

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

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

83 Group 3(200mg/kgbw OG) -Induction with 8% NaCl for 4weeks and subsequent treatment with
84 200mg/kgbw OG for another 4 weeks.

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

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

89 **2.4 Blood pressure measurement in rats using Kent Scientific CODA machine**

90 Volume pressure recording (VPR) method of blood pressure measurement was used as suggested
91 by the American Heart Association¹⁰. Blood pressure was measured using VPR non-invasive
92 blood pressure monitoring system (CODA-6) Kent Scientific, Torrington, CT).

93 **Blood pressure measurement procedure**

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

101 **2.5 LD 50: Acute toxicity studies of methanolic extract of *Ocimum gratissimum*(MEOG)**

102 The acute toxicity of MEOG was determined by Lorke's method¹¹ as described by Enegide¹².
103 Rats were divided into two phases. In the first phase of the study, 9 rats were divided into 3
104 groups of 3 rats each and they were treated with MEOG through oral gavage at the doses of 10,
105 100 and 1000 mg/kg respectively. The rats were observed for behavioural change and Lethality
106 in the first 24 hrs.

107 In the second stage, 3 rats were divided into 3 groups of 1 rat each and they were treated with
108 MEOG through oral gavage at the doses of 1600, 2900 and 5000mg/kg. The general behavior of
109 the animals were observed continuously for 1 hr after treatment and then intermittently for 4 hrs,
110 then hourly for the next 24hrs. The LD₅₀ was determined using the formular

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

111 Where a = minimal lethal dose

112 $b = \text{maximal survival dose}$

113 $OG LD_{50} = \sqrt{a \times b} = \sqrt{1600 \times 5000} = > \sqrt{8000000} = 2828$

114 **2.6 Biochemical methods/assay**

115 **Determination of Alkaloids¹³**

116 Alkaloids were determined using the method of Harborne, 1973.

117 **Procedure:**

118 Five grams (5g) of the sample was weighed into a 250ml beaker and 200ml of 20% acetic acid in
 119 ethanol was added and covered and allowed to stand for 4 hours at 25°C. This was filtered with
 120 filter paper No. 42 and the filtrate was concentrated using a water bath (Memmert) to one quarter
 121 of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract
 122 until the precipitate was complete. The whole solution was allowed to settle and the precipitate
 123 was collected and washed with dilute NH₄OH (1% ammonia solution). Then, filter with pre-
 124 weighed filter paper. The residue on the filter paper is the alkaloid, which is dried in the oven
 125 (precision electrothermal model BNP 9052 England) at 80°C. The alkaloid content was
 126 calculated and expressed as a percentage of the weight of the sample analyzed.

127 **Calculation:**

128 $\% \text{weight of alkaloid} = \frac{\text{weight of filter paper with residue} - \text{weight of filter paper}}{\text{Weight of sample analyzed}} \times 100$
 129

130 **Determination of Flavonoids¹⁴**

131 Flavonoids were determined using the method of Bohm and Kocipai-Abyazan, 1994.

132 **Procedure:**

133 10g of the plant sample was extracted repeatedly with 100ml of 80% aqueous methanol at room
 134 temperature. The whole solution was filtered through whatman filter paper No. 42 (125mm).

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

137 **Calculation:**

$$\begin{aligned} 138 \quad \% \text{flavonoids} = & \quad \frac{(\text{weight of crucible} + \text{residue}) - (\text{weight of crucible})}{\text{Weight of sample analyzed}} \times 100 \\ 139 \end{aligned}$$

140 **Determination of Saponin**¹⁵

141 Saponin was determined using the method of Obadoni and Ochuko, 2001.

142 **Procedure:**

143 20 g of each grounded sample was put into a conical flask and 100 cm³ of 20% aqueous ethanol
144 was added. Then the flask was heated on a hot water bath for 4 hrs with constant stirring at about
145 55°C. The mixture was then filtered and the residue was again extracted with another 200 ml
146 20% ethanol. The combined extract was reduced to 40 ml on a hot water bath at about 90°C. The
147 concentrate was transferred into a 250 ml separatory funnel, added 20 ml diethyl ether in it
148 followed by vigorous shaking. The aqueous layer was recovered while the ether layer was
149 discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined
150 n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining
151 solution was heated in a water bath. After evaporation the samples were dried in oven, weighed
152 and saponin content was calculated as percentage

153 **Calculation:**

$$\begin{aligned} 154 \quad \% \text{ saponin content} = & \quad \frac{(\text{weight of filter paper} + \text{residue}) - (\text{weight of filter paper})}{\text{Weight of sample analyzed}} \times 100 \\ 155 \end{aligned}$$

156 **Determination of Tannin**¹⁶

157 Tannin content of the sample was determined by Follins Dennis titration method as described by
158 Pearson, 1974.

159 **Procedure:**

160 The follins Dennis titrating method as described by Pearson (1974) was used. To 20g of the
161 crushed sample in a conical flask was added 100mls of petroleum ether and covered for 24

162 hours. The sample was then filtered and allowed to stand for 15 minutes allowing petroleum
163 ether to evaporate. It was then re-extracted by soaking in 100ml of 10% acetic acid in ethanol for
164 4hrs. The sample was then filtered and the filterate collected. Then 25ml of NH_4OH were added
165 to the filterate to precipitate the alkaloids. The alkaloids were heated with electric hot plate to
166 remove some of the NH_4OH still in solution. The remaining volume was measured to be 33ml.
167 5ml of this was taken and 20ml of ethanol was added to it. It was titrated with 0.1 M NaOH
168 using phenolphthalein indicator until a pink end point is reached.

169 **Calculation:**

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

171 Where:

172 C_1 = conc. of Tannic Acid

173 C_2 = conc. Of Base

174 V_1 = Volume of Tannic acid

175 V_2 = Volume of Base

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

177 V_1

178 % of tannic acid content = $\frac{C_1 \times 100}{\text{Weight of sample analyzed}}$

180 **Determination of Total Phenols**¹⁷

181 Total phenols were determined by spectrophotometric method as described by Khan et al., 2011.

182 **Procedure:**

183 Two grams (2 g) of each plant sample was defatted with the help of 100 ml of diethyl ether using
184 a soxhlet apparatus for 2 h. The fat free sample was boiled with 50 ml of ether for 15 min for the
185 extraction of phenolic component. 5 ml of the extract was pipetted into a 50 ml flask and 10 ml
186 distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated

187 amylalcohol were also added in it. The samples were made up to mark and left to react for 30
188 min. Colour was developed and its absorbance was measured at 505 nm.

189 **Determination of Phytate Contents**¹⁸

190 Phytate contents were determined using the method of Lucas and Markakes (1975).

191 **Procedure:**

192 0.2g of each samples was weighed into different 250ml conical flasks. Each sample was soaked
193 in 100 ml of 2% concentrated HCl for 3 hours. The samples were then filtered. 50 ml of each
194 filtrate was placed in 250ml beaker and 100ml distilled water added to each sample. 10 ml of
195 0.3% ammonium thiocyanate solution was added as indicator and titrated with standard iron (III)
196 chloride solution which contained 0.00195g iron per 1 ml.

197 **Calculation:**

$$198 \text{ Phytic acid} = \frac{\text{Titre value} \times 0.00195 \times 1.19 \times 100}{\text{Wt of sample}}$$

200 **Determination of mineral content of *Ocimum gratissimum***

201 Mineral content was determined by Association of Official Analytical Chemists methods¹⁹ using
202 the flame system of the atomic absorption spectrophotometry (AAS), (Varian Spectr AA240,
203 USA).

204 **Procedure:**

205 *Ocimum gratissimum* was ashed at 550°C overnight and the ash was dissolved in concentrated
206 nitric acid and filtered, diluted to 50 ml with deionized water and the absorbance of the samples
207 was read directly on the AAS. Working standard solutions of potassium, calcium, magnesium,
208 phosphorus and sodium were prepared from stock standard solution (1000 ppm), in 2 N HNO₃
209 and absorbance was noted for standard solution of each element and samples using atomic
210 absorption spectrophotometer (AAS). Calibration curve for each metal was prepared by plotting
211 the absorbance of standards versus their concentrations. A blank reading was also taken and
212 necessary corrections were made during the calculation of concentration of various elements.

213 Determination of Urea Level ²⁰

214 Blood urea was determined by the method described by Kassirer, (1971).

215 Principle

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

220 Procedure:

221 Test tubes were labelled: reagent blank, Standard, Control and Sample. 1.0 ml of enzyme reagent
222 (urease, ADP), followed by 0.01 ml of sample/standard/control in their respective tubes and
223 mixed thoroughly. These solutions were allowed to stand at 37°C for one minute. 0.250 ml of 2-
224 oxoglutarate and NADH reagent was added to all the tubes and mixed. The initial absorbance
225 was measured after 30 seconds (A1) and the second reading taken at exactly one minute (A2)
226 measured against the reagent blank at 340 nm.

227 Calculation:

228 The mean change in absorbance readings were calculated thus ($\Delta A2-A1/\text{min}$)

$$229 \text{ Urea (mmol/l)} = \frac{\Delta A_{\text{sample}}}{\Delta A_{\text{std}}} \times \text{Concentration of standard}$$

230

231 3.5.13 Determination of Creatinine Level ²¹

232 Serum creatinine was estimated by Jaffe's method as described by Laron, (1972).

233 Principle

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

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

238 **Procedure:**

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

246 **Calculation:**

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

248 $\Delta\text{Abs. of standard}$

249 **Determination of CK Activity²²**

250 CK activity was assayed using the methods of Szasz, (1977) as recommended by the IFCC.

251 **Principle**

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

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

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

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

257 **Creatine kinase-MB determination²³**

258 Immunoinhibition method was used in estimation of CK-MB. The sample was incubated in the
 259 CK-MB reagent which includes the CK-M antibody. The activity of the non-inhibited CK-B was
 260 then determined using the following series of reaction. CK-B catalyses the reversible

261 phosphorylation of ADP, in the presence of creatine phosphate to form ATP and creatinine. The
262 auxillary enzyme hexokinase (HK) catalyses the phosphorylation of glucose by ATP format, to
263 produce ADP and glucose-6-phosphate is oxidized to 6-phosphogluconate with concomitant
264 production of NADH. The rate of NADH formation, measured at 340nm, is directly proportional
265 to serum CK-B activity.

266 **2.7 Statistical Analysis**

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

273

274 **3.0 RESULTS**

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

278 Systolic blood pressure, diastolic blood pressure, serum sodium, chloride, urea and creatinine
279 were significantly increased ($p<0.05$) while serum potassium and bicarbonate were significantly
280 decreased ($p<0.05$) after 4 weeks induction of hypertension. After administration of
281 methanolic extract of *Vernonia amygdalina* and *Ocimum gratissimum* at 200 mg/kgbw and
282 400mg/kgbw systolic blood pressure, diastolic blood pressure, serum sodium, chloride, urea
283 and creatinine were significantly decreased ($p<0.05$) while serum potassium significantly
284 increased ($p<0.05$) after 4 weeks induction of hypertension in a dose-dependent manner(table
285 2-9). Serum creatine and creatine-kinase did not differ significantly at 4 weeks and 8 weeks
286 respectively (table 10-11).

287

288 4.0 DISCUSSION

289 Hypertension was induced in male wistar rats to look at the possible prevention and management of
290 hypertension using methanolic extract of *Ocimum gratissimum*(OG) in comparison with a reference
291 drug, lisinopril. Induction of hypertension was achieved through oral gavage administration of 8% NaCl
292 in water for a period of 4 weeks. The induction process was in agreement with work done by Rini²⁴. The
293 oral dosing of 8% NaCl was performed without anaesthesia. The NaCl induced hypertension may have
294 been achieved through increased sympathetic nerve activity, a major trigger of vasoconstriction as well
295 as the activation of renin angiotension aldosterone system²⁵. Phytochemical analysis revealed the
296 presence of steroid, flavonoids, phenol, alkaloids, saponin, tannis and phytate while mineral content
297 revealed the presence of potassium, magnesium, phosphorus, calcium and sodium. The phytochemical
298 result obtained was in accordance with Udochukwu²⁶. After 4 weeks, systolic and diastolic blood
299 pressures were significantly elevated in all the groups except group 1 which is the control group. On
300 treatment with 200mg/kg OG, 400mg/kgOG and 30mg/kg lisinopril, the elevated SBP and DBP
301 significantly reduced ($P<0.05$). The significant reduction in SBP and DBP may be related to the high
302 concentration of potassium and magnesium in methanolic extract of *Ocimum gratissimum*. Potassium
303 and magnesium are important for muscle function, which includes relaxing the walls of blood vessels.
304 Normal potassium levels are important for the conduction of electrical signals in the nervous system and
305 in the heart. This protects against an irregular heartbeat. Potassium counters the effect of sodium
306 which was significantly increased after 4 weeks induction with NaCl. At 8 weeks, serum sodium level was
307 significantly reduced and potassium was significantly increased. Urea and creatinine were significantly
308 increased at 4weeks suggesting impairment of renal function or loss of structural integrity of the kidney
309 cell membrane but at 8weeks, the elevated renal parameters were reduced significantly. The observed
310 changes is in accordance with the report of Ogundipe²⁷ who reported on the effects of two
311 weeks administration of *Ocimum gratissimum* leaf on feeding pattern and markers of renal
312 function in rats treated with gentamicin. Ogundipe observed a decrease in urea and creatinine
313 although it was for a shorter period of 2 weeks. Reduction in the renal markers suggests that
314 methanolic extract of *Ocimum gratissimum* supplementation may lead to increase in glomerular
315 filtration rate hence maybe helpful in hypertensive nephropathy. Methanolic extract of *Ocimum*
316 *gratissimum* did not significantly affect the serum levels of creatine kinase(CK)and creatine kinase-MB
317 which makes the cardioprotective effect of the extract uncertain in this study.

318 5.0 CONCLUSION

319 *Ocimum gratissimum* possesses antihypertensive effect and enhances the proper functioning of
320 the kidney. It may also be useful in hypertensive condition due to its nephroprotective effect at
321 200mg/kgbw and 400 mg/kgbw.

322

10.0 References

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11.0 TABLES

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

| PHYTOCHEMICAL(mg/100g) | <i>Ocimum gratissimum</i> (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 (HCO₃) 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($\mu\text{mol/l}$)n=8 | 0 week | 4 weeks | 8 weeks |
|--|------------------|-------------------------------|------------------------------------|
| Neg control | 47.18 \pm 1.49 | 47.17 \pm 1.47 | 47.16 \pm 1.47 |
| Untreated group | 47.67 \pm 1.21 | 63.35 \pm 3.77 ^a | 61.17 \pm 4.95 ^{b,*} |
| OG 200mg/kg bwt | 47.16 \pm 1.47 | 62.00 \pm 5.32 ^a | 51.33 \pm 5.71 ^{b,c,*} |
| OG 400mg/kg bwt | 47.33 \pm 1.21 | 63.33 \pm 1.03 ^a | 49.50 \pm 3.72 ^b |
| Lisinopril 30mg/kg bwt | 47.00 \pm 0.89 | 66.00 \pm 2.75 ^a | 68.50 \pm 13.40 ^{b,c,*} |

Values expressed as mean \pm 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 \pm 0.09 | 44.63 \pm 1.27 | 44.49 \pm 1.83 |
| Untreated group | 45.10 \pm 0.17 | 44.95 \pm 1.75 | 46.99 \pm 1.54 |
| OG 200mg/kg bwt | 45.37 \pm 0.07 | 44.39 \pm 0.92 | 43.58 \pm 1.21 |
| OG 400mg/kg bwt | 45.13 \pm 0.24 | 43.92 \pm 0.95 | 43.72 \pm 0.66 |
| Lisinopril 30mg/kg bwt | 44.97 \pm 0.94 | 43.82 \pm 1.08 | 43.48 \pm 1.36 |

Values expressed as mean \pm 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.