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Phytochemical, Mineral Analysis and Antioxidative Effect of Methanolic Extract of *Ocimum gratissimum* in Hypertensive Male Wistar Rats.

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ABSTRACT

Hypertension is a serious public health problem associated with oxidative stress which can damage cells and may also play a role in development of neurodegenerative diseases. *Ocimum gratissimum* is a medicinal plant with useful phytochemicals and minerals. Antioxidative effect of methanolic extract of *Ocimum gratissimum* (MEOG) in tissue samples of hypertensive male wistar rats was investigated. Forty wistar rats (100-110)g were assigned to five groups of eight rats each. Group 1-5 constitutes the normal, hypertensive group, MEOG (200 mg/kg bwt) group, MEOG (400 mg/kg bwt) and reference drug (lisinopril, 30 mg/kg) group respectively. The extract and reference drug were given through oral gavage. All groups except group 1 were induced with 8% NaCl from 0-4weeks before treatment with extract and reference drug from 5-8 weeks. At 8weeks, the tissue samples (liver, kidney, testes and heart) were excised out and homogenized for analysis. Phytochemical, mineral and oxidative stress markers analysis was done using standard methods. The mean level of antioxidant enzymes on tissue samples of liver, kidney, testes and heart showed significant ($P<0.05$) increase at different doses when compared with the control group. Moreover, at higher doses of 400mg/kg/bwt of MEOG, there was significant increase when compared to 200mg/kg/bwt of MEOG. In lisinopril group and untreated group, there were significant decrease ($P<0.05$) when compared with the control group at 8weeks. The mean level of Malondialdehyde (MDA) on tissue samples of liver, kidney, testes and heart showed significant ($P<0.05$) increase in untreated group, lisinopril group and 200mg/kg/bwt of MEOG when compared with the control group. Moreover, at higher doses of 400mg/kg/bwt of MEOG, there was significant ($P<0.05$) decrease when compared with the control group. *Ocimum gratissimum* possesses antioxidative effect which may be linked to the rich phytochemicals and minerals resident in the plant. Therefore, may be beneficial in ameroliating the effect of oxidative stress in hypertensive condition.

Keywords: Hypertension, Methanolic extract of *Ocimum gratissimum* (MEOG), Oxidative stress markers, Malondialdehyde (MDA).

INTRODUCTION

Hypertension is a serious public health problem associated with oxidative stress which can damage cells and may also play a role in development of neurodegenerative diseases. *Ocimum gratissimum* 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. It is used as vegetable in meal preparation. The leaf extract of *Ocimum gratissimum* showed anti-diabetic properties [1,2], antibacterial activities [3,4], anti tumour and anti-cancer effects [5], Mosquito repellent and mosquitocidal potential [6] and hepatoprotective effect [7,8]. *Ocimum gratissimum* is a medicinal plant with useful phytochemicals and minerals.

Aim and objective of the study

Antioxidative effect of methanolic extract of *Ocimum gratissimum* (MEOG) in tissue samples of hypertensive male wistar rats was investigated. The phytochemical and mineral analysis of *Ocimum gratissimum* were also investigated.

METHODOLOGY

Forty wistar rats (100-110)g were assigned to five groups of eight rats each. Group 1-5 constitutes the normal, hypertensive group, MEOG (200 mg/kg bwt) group, MEOG (400 mg/kg bwt) and reference drug (lisinopril, 30 mg/kg) group respectively. The extract and reference drug were given through oral gavage. All groups except group 1 were induced with 8% NaCl from 0-4weeks before treatment with extract and reference drug from 5-8 weeks. At 8weeks, the tissue samples (liver, kidney, testes and heart) were excised out and homogenized for analysis.Phytochemical, mineral and oxidative stress markers analysis were done using standard methods.

Methanolic extraction of the leaves- Maceration technique.

A bag of scent leaf was air-dried at room temperature. Air-dried leaves of the plant were milled into powder using a blender-pyramid brand with model number PM-Y44B3.

The powdered leaves were weighed and macerated (soaked) in methanol (500g of the plant material to 2.5liters of methanol in a stopper) for 3 days with frequent agitation to soften and break the plant cell wall to ensure sufficient extraction of the active phytochemicals [9]. At the end of three days, the methanolic extracts were filtered using whatman No.1 filter paper and the filtrate concentrated to dryness under reduced pressure at 60^0 C in a rotary evaporator at 45^o C, weighed and stored frozen until use. The exact weight of dried extract from 500g powder was 87.8g gave a percentage yield of 17.56% for *Ocimumgratissimum*. The extracts were dissolved and were given to the animals through oral gavage at graded doses of 200mg/kg body weight and 400mg/kg body weight. The choice of dosage of *Ocimumgratissimum* was according to [10], who worked on graded doses (200mg/kg and 400mg/kg) of methanolic extract of *Ocimum gratissimum*.

Determination of SOD activity

SOD was assayed by colorimetric method of Misra and Fredovich [11]

Principle:

The ability of superoxide dismutase to inhibit the auto oxidation of adrenaline at pH 10.2 makes this reaction a basis for the SOD assay. Superoxide anion (O_2^-) generated by the xanthine oxidase reaction is known to cause the oxidation of adrenaline to adrenochrome. The yield of adrenochrome produced per superoxide anion introduced increased with increasing pH and also with increasing concentration of adrenaline. These led to the proposal that auto oxidation of adrenaline proceeds by at least two distinct pathways, one of which is a free radical chain reaction involving superoxide radical and hence could be inhibited by SOD. See appendix for reagents, procedure and calculation.

Procedure:

Eighty (80 μl) of sample/blank was added into a clean test tube containing 1000 μl of carbonate buffer (pH 10.2). The resulting solution was mixed thoroughly, and allowed to equilibrate by incubating at 37°C for 5 minutes. Thereafter, 600 μl of freshly prepared epinephrine was added and the reaction mixture was read at 30 seconds interval for 150 seconds at 480 nm. The blank was treated the same way except that 80 μl of distilled water was used instead of plasma. The changes in absorbances of both test and blank were determined. The % inhibition of auto oxidation of epinephrine by SOD was calculated and the plasma SOD activity was expressed as U/ml. One unit of SOD activity was equivalent to the amount of

SOD that can cause 50% inhibition of epinephrine.

Calculation:

% inhibition = (ΔOD_{blank} - ΔOD_{test} / ΔOD_{blank}) X 100

Enzyme Unit (U/ml) = (% inhibition/50) X dilution factor.

Determination of catalase Activity

Catalase activity was determined by colorimetric method of Sinha [12].

Principle

This method is based on the fact that dichromate in acetic acid is reduced to chromic acetate when heated in the presence of H_2O_2 with the formation of perchromic acid as an unstable intermediate. The chromic acetate then produced is measured colorimetrically at 570 to 610nm. Since dichromate has no absorbance in this region, the presence of the compound in the assay mixture does not interfere at all with the colorimetric determination of chromic acetate. The catalase preparation is allowed to split H_20_2 for different periods of time. The reaction was stopped by the addition of dichromate acetic acid mixture and the remaining H_2O_2 is determined by measuring chromic acetate colorimetrically after heating the reaction mixture.

Procedure:

One milliliter (1 ml) of phosphate buffer (pH 7.0) was mixed with 0.4 ml of hydrogen peroxide and allowed for some time to equilibrate at room temperature. 0.1 ml of sample was added into the reaction mixture and was gently swirled at room temperature. 2 ml dichromate/acetic acid reagent was added after one-minute interval and was thoroughly mixed. The solution was incubated in boiling water for 10 minutes and allowed to cool. The sample blank was prepared and treated the same way except that dichromate/acetic acid mixture was added before the addition of the sample. The colour developed was read at 570 nm and the activity of catalase in U/ml which is equivalent to the amount of hydrogen peroxide (μmol) degraded per minute was calculated using 20 and 40 μmol of hydrogen peroxides as standards.

Calculation:

Activity of catalase (U/ml) = OD_{blank} - OD_{test} /OD_{std} X Std concentration.

Determination of GST Activity

GST activity was determined spectrophotometrically at 37°C as described by [13].

Principle

Glutathione-s-transferase catalyses the conjugation of reduced glutathione with the aromatic substrate 1-chloro-2, 4-dinitrobenzene to form a complex. The complex formed has a characteristic absorption at 340nm. The spectrophotometric readings are considered indicative of enzyme activity.

Procedure:

The GST reagent was initially prepared by mixing 50 ml of phosphate buffer (pH 6.5) with 2 ml of CDNB and allowed to equilibrate at room temperature (25°C) for at least 10 minutes. Thereafter, 0.8 ml of GST reagent was mixed with 0.1 ml of reduced glutathione. The reaction was started by adding 0.1 ml of sample and the absorbance at 340 nm was read for 3 minutes at 1minute interval. The blank was treated the same way except that distilled water was used instead of the sample.

Calculation:

$$GST\ (U/L) = \frac{\Delta Ab \times TV \times 1000}{E_{340} \times SV}$$

Were:

ΔAb = change in absorbance of the test

E_{340} = Molar extinction coefficient for GST ($9.6\ M^{-1}cm^{-1}$)

TV = Total volume of reaction mixture

SV = Volume of sample.

Determination of MDA level

MDA level was determined by the colorimetric method of [14].

Principle:

Malondialdehyde (MDA) is a product of lipid peroxidation. When heated with 2-thiobarbituric acid (TBA) under alkaline condition, it forms a pink coloured product, which has absorption maximum at 532 nm. The intensity of colour generated is directly proportional to the concentration of MDA in the sample.

Procedure:

To 0.1 ml of sample in test tube was added 1 ml of 1% thiobarbituric acid dissolved in alkaline medium (sodium hydroxide). The mixture was mixed thoroughly, and 1 ml of glacial acetic acid was added to the mixture. The reaction mixture was also shaken thoroughly and incubated in boiling water (100^0C) for 15 minutes. It was allowed to cool and the turbidity removed by centrifugation at 3000 rpm for 10 minutes. Thereafter, the supernatant was read at 532 nm. The same volume of TBA and glacial acetic acid was added to the blank, but 0.1 ml of distilled water was added to the blank instead of plasma. The level of MDA in the serum is expressed as nmol/lml using the molar extinction coefficient for MDA ($1.56x\ 105\ M^{-1}cm^{-1}$).

Determination of Total antioxidant capacity

Total antioxidant activity was estimated by ferric reducing ability of plasma (FRAP) method by [15].

At low pH, antioxidant power causes the reduction of ferric tripyridyl triazine (FeIII TPTZ) complex to ferrous form (which has an intense blue colour) that can be monitored by measuring the change in absorbance at 573nm. FRAP values are obtained by comparing the absorbance change at 573nm in mixture (test), with those containing ferrous ion in known concentration (standard).

Calculation:

MDA (nmol/ml) = (OD X 1000000)/E_{532}

Where E_{532} = Molar extinction coefficient for MDA ($1.56x10^5$ as $M^{-1}cm^{-1}$)

RESULTS

The mean level of antioxidant enzymes on tissue samples of liver, kidney, testes and heart showed significant ($P<0.05$) increase at different doses when compared with the control group. Moreover, at higher doses of 400mg/kg/bwt of MEOG, there was significant increase when compared to 200mg/kg/bwt of MEOG. In lisinopril group and untreated group, there were significant decrease ($P<0.05$) when compared with the control group at 8weeks. The mean level of Malondialdehyde (MDA) on tissue samples of liver, kidney, testes and heart showed significant ($P<0.05$) increase in untreated group, lisinopril group and 200mg/kg/bwt of MEOG when compared with the control group. Moreover, at higher doses of 400mg/kg/bwt of MEOG, there was significant ($P<0.05$) decrease when compared with the control group.

Table 1 Phytochemical and mineral component of methanolic extract of *Ocimum gratissimum*.

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 Tissue malondialdehyde (MDA) after NaCl induced hypertension and following treatment with MEOG and Lisinopril in wistar rats after 8 weeks (mean± S.D).

MDA(nmol/l) n=8	Liver	Kidney	Testes	Heart
Neg control (G1)	0.63 ±0.01	0.75±0.02	0.62±0.11	0.52±0.01
Untreated group (G2)	3.71±0.02	4.41±0.01	3.51±0.01	3.37±0.22
OG 200mg/kg bwt(G3)	2.36±0.05	2.93±0.03	1.09±0.02	1.14±1.15
OG 400mg/kg bwt(G4)	0.61±0.01	0.65±0.01	0.62±0.02	0.54±0.02
Lisinopril 30mg/kg bwt(G5)	3.71±0.08	4.39±0.45	3.47±0.08	3.34±0.25
Fvalue	15144.280	18169.648	7225.307	475.257
Pvalue	0.001	0.001	0.001	0.001
Post hoc 1 vs2	P=0.001	P=0.001	P=0.001	P=0.001
Post hoc 1vs3	p=0.032	p=0.022	p=0.032	p=0.012
1vs4;5	p=0.001;0.001	p=0.001;0.001	p=0.001;0.001	p=0.001;0.001
3vs4	P=0.001	P=0.001	P=0.001	P=0.001

Key: MDA=malondialdehyde. P –value< 0.05 is considered statistically significant.

Table 3 Tissue Superoxide dismutase (SOD) after NaCl induced hypertension and following treatment with MEOG and Lisinopril in wistar rats after 8 weeks (mean± S.D).

SOD(U/l) n=8	Liver	Kidney	Testes	Heart
Neg control (G1)	11.45±0.11	10.59±0.26	9.48±0.14	11.48±0.28
Untreated group (G2)	7.38±0.32	7.33±0.23	5.44±0.21	6.58±0.27
OG 200mg/kg bwt(G3)	12.44±0.21	13.42±0.19	12.34±0.25	11.31±0.14
OG 400mg/kg bwt(G4)	18.14±0.50	17.52±0.07	16.21±0.46	15.49±0.08
Lisinopril 30mg/kgbwt(G5)	9.56±0.16	9.14±0.02	8.12±0.02	8.39±0.07
Fvalue	1034.230	1365.990	1094.157	1638.569
Pvalue	0.001	0.001	0.001	0.001
Post hoc 1 vs2	P=0.001	P=0.001	P=0.001	P=0.001
1vs3	p=0.044	p=0.040	p=0.024	p=0.030
1vs4;5	p=0.001;0.001	p=0.001;0.001	p=0.001;0.001	p=0.001;0.001
3vs4	P=0.001	P=0.001	P=0.001	P=0.001

Key: SOD- superoxide dismutase. P –value< 0.05 is considered statistically significant.

Table 4 Tissue Glutathione-S- transfarase (GST) after NaCl induced hypertension and following treatment with MEOG and Lisinopril in wistar rats after 8 weeks (mean± S.D).

GST(u/l) n=8	Liver	Kidney	Testes	Heart
Neg control (G1)	5.30±0.08	5.52±0.14	4.85±0.30	5.46±0.23
Untreated groupl (G2)	3.76±0.10	3.55±0.21	3.06±0.12	3.16±0.27
OG 200mg/kg bwt(G3)	9.17±0.13	10.30±0.17	7.46±0.08	8.61±0.18
OG 400mg/kg bwt(G4)	14.58±0.23	12.63±0.08	9.41±0.11	11.32±0.12
Lisinopril 30mg/kgbwt(G5)	4.18±0.11	4.80±0.15	3.56±0.12	3.11±0.28
Fvalue	2821.499	3693.347	1933.444	1393.342
Pvalue	0.001	0.001	0.001	0.001
Post hoc 1 vs2	P=0.001	P=0.001	P=0.001	P=0.001
1vs3	p=0.004	p=0.040	p=0.022	p=0.010
1vs4;5	p=0.001;0.001	p=0.001;0.001	p=0.001;0.001	p=0.001;0.001
3vs4	P=0.001	P=0.001	P=0.001	P=0.001

Key: GST-glutahione -S- transferase. $P < 0.05$ = considered statistically significant.

Table 5 Tissue Catalase (CAT) after NaCl induced hypertension and following treatment with MEOG and Lisinopril in wistar rats for 0-8 weeks (mean± S.D).

CAT(u/ml) n=8	Liver	Kidney	Testes	Heart
Neg control (G1)	43.33±1.86	42.80±1.99	28.70±0.77	37.40±1.33
Untreated group (G2)	22.16±0.50	21.30±0.67	16.05±0.69	29.88±0.82
OG 200mg/kg bwt(G3)	45.03±1.07	47.90±0.69	38.46±2.17	39.60±0.83
OG 400mg/kg bwt(G4)	48.73±1.36	52.61±0.98	32.20±1.41	42.70±1.20
Lisinopril 30mg/kg bwt(G5)	42.50±1.37	41.43±1.21	27.28±0.90	30.55±1.65
Fvalue	199.437	485.876	197.837	183.934
Pvalue	0.001	0.001	0.001	0.001
Post hoc 1 vs2	P=0.001	P=0.001	P=0.001	P=0.001
1vs3	p=0.003	p=0.040	p=0.021	p=0.024
1vs4;5	p=0.001;0.001	p=0.001;0.001	p=0.001;0.001	p=0.001;0.001
3vs4	P=0.001	P=0.001	P=0.001	P=0.001

Key: CAT=catalase. P –value< 0.05 is considered statistically significant.

Table 6 Tissue total antioxidant capacity (TAC) after NaCl induced hypertension and following treatment with MEOG and Lisinopril in Wistar rats after 8 weeks (mean± S.D).

TAC(u/l) n=8	Liver	Kidney	Testes	Heart
Neg control (G1)	184.76±1.29	193.60±5.27	164.56±4.75	94.76±1.46
Untreated group(G2)	167.45±4.59	130.78±4.77	123.83±1.64	42.76±4.2
OG 200mg/kg bwt(G3)	378.42±2.44	270.50±1.43	296.68±4.05	57.52±2.43
OG 400mg/kg bwt(G4)	398.17±3.49	292.35±6.98	314.40±7.90	63.81±4.63
Lisinopril 30mg/kg bwt(G5)	173.80±0.76	197.06±2.79	164.36±3.12	86.26±5.49
Fvalue	10828.83	1672.131	1748.74	196.71
Pvalue	0.001	0.001	0.001	0.001
Post hoc 1 vs2	P=0.001	P=0.002	P=0.001	P=0.002
1vs3	p=0.004	p=0.003	p=0.001	p=0.001
1vs4;5	p=0.001;0.001	p=0.001;0.001	p=0.001;0.001	p=0.001;0.001
3vs4	P=0.001	P=0.001	P=0.001	P=0.001

DISCUSSION

The result of this study revealed that *Ocimum gratissimum* contains different phytochemicals and minerals as seen in Table 1. The marker of lipid peroxidation, MDA significantly increased after induction of hypertension. The increase in MDA persisted in hypertensive control group (Group 2). After treatment with *Ocimum gratissimum*, the serum level of MDA significantly decreased but not in reference group (group 5). The result of the MDA level corroborates with the report of [16], who reported a decrease in MDA level in the study on chemoprotective effect of *Vernonia amygdalina* (Astereacea) against 2-acetyl amino fluorine-induced hepatotoxicity in rats. The result of the work is also in agreement with [17] who reported that alteration in blood pressure, antioxidant status and caspase 8 expressions in cobalt-induced cardio-renal dysfunction were reversed by *Ocimum gratissimum* and gallic acid in wistar rats. Likewise, the result of the study from *invivo Vernonia amygdalina and Ocimum gratissimum* on oxidative stress indicators in rats showed that the leaves have potentials to be valuable in reducing powers and free radical quenching [18]. Similarity of the result obtained from MDA is also in accordance with [19], who showed that aqueous leaf content of *Ocimum gratissimum* decreased MDA. Malondialdehyde (MDA) is the principal and most studied product of polyunsaturated fatty acid peroxidation. It is a highly toxic molecule and its interaction with DNA and proteins has often been referred to as potentially mutagenic and atherogenic [20]. Lipid peroxidation is a chain reaction and is created by free radicals influencing unsaturated fatty acids in cell membrane, leading to their damage. Free radicals are initiators of peroxidation processes. Once

activated, reaction continues autocatalytically which have a progressive course and finally result is structural and functional changes of substrate. Induction of ROS and lipid peroxidation can lead to important changes in membrane function such as the modification of transmembrane potential (depolarization), release of mitochondrial calcium and division, and activation of caspases-3, DNA fragmentation, and apoptosis [21].

Increase in MDA has been associated with hypertension [22]. MDA has been shown not to be the cause but the effect of hypertension [23].*Ocimum gratissimum* showed a reduction in MDA levels but lisinopril treated group did not show a reduction in MDA level. Reduction of MDA in *Ocimum gratissimum* treated group may be linked to the phytochemical content and free radical quenching ability resulting in a reduction of blood pressure could help in preventing the complications of hypertension, mediated by the free radicals.

The result of the antioxidant parameters (SOD, CAT, GST, and TAC) showed a significant decrease after induction of hypertension but significantly increased after treatment with *Ocimum gratissimum*. The lisinopril treated group did not show a significant decrease in antioxidant enzyme activity after treatment. This is in accordance with other researchers [16, 17] who reported an increase activity in antioxidant enzymes (GST, SOD, CAT) after administration of *Ocimum gratissimum*. Likewise, study from aqueous leaf extract of *Ocimum gratissimum* potentials activities of plasma and hepatic antioxidant enzymes in rats showed a significant increase in dose dependent pattern while GST showed no significance when compared with control [24]. The disparity in GST result may be related to the aqueous solvent used in the extraction of the leaves that are not able to dissolve some of the useful phytochemicals that are resident the plant leaves. Other researchers also showed concordance with the study [19, 25]. After induction of hypertension, an imbalance in the redox system was generated due to the increase in lipid peroxidation and decrease in antioxidant parameters (SOD, GST, TAC, CAT). On treatment with the methanolic extract of *Ocimum gratissimum*, the MDA decreased and the antioxidant enzymes increased showing that *Ocimum gratissimum* may be rich in antioxidants. The redox imbalance resulted to oxidative stress which was generated as a result of hypertension [26]. The presence of phytochemicals (flavonoids and phenols) residents in *Ocimum gratissimum* are good source of antioxidants, scavenging the body for unstable cellular molecules that cause DNA damage. These phytochemicals also help to inhibit the progression of chronic diseases such as cardiovascular disease, cancer and age-related vascular degeneration and decrease inflammation by decreasing the binding of inflammatory markers. The alkaloid helps to remove toxins from the blood and decrease calcium deposits in blood vessels helping decrease the risk of cardiovascular disease.

CONCLUSION

Ocimum gratissimum possesses antioxidative effect which may be linked to the rich phytochemicals and minerals resident in the plant. Therefore, may be beneficial in ameroliating the effect of oxidative stress in hypertensive condition.

Declaration of interest: None

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