IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN: 2278-3008, p-ISSN:2319-7676. Volume 8, Issue 3 (Nov. – Dec. 2013), PP 01-05
www.iosrjournals.org

Effects of NaCl and Lime water on the Hypoglycemic and Antioxidant activities of *Ocimum gratissimum* in Alloxan-induced Diabetic rats.

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Abstract: *The antidiabetic and antioxidant activities of Ocimum gratissimum (O.G) are well documented. This study was designed to investigate the effects of adding NaCl or lime water to aqueous leaves extract of O.G in al loxan (100mg/kg)-induced diabetic rats. 25 male wistar rats of average weight 180 were divided into 5 groups a s; control, diabetic, diabetic treated with O.G (400 mg/kg), diabetic treated with O.G+ NaCl and diabetic treate d with O.G + lime water for six weeks. At the end of the experiment, plasma glucose level, Total protein, Albumi n and liver ALT, AST, MDA and GSH were determined. The results showed that the aqueous extract of O.G, sig nificantly (P<0.05) reduced the plasma glucose, liver MDA and significantly (P<0.05) increased the liver GSH and ALT of the treated diabetic rats. However these values were found to be significantly (P<0.05) reduced by t he addition of NaCl and limewater. While there were no significant (P<0.05) differences in the plasma albumin, total proteins and liver AST in the treated groups as compared with the control. This study showed that the addi tion of NaCl or lime water to the extracts of O. gratissimum may reduce the hypoglycemic and antioxidant activi ties associated with O. gratissimum.*

Key words: *Antidiabetic, Antioxidant, Lime water, NaCl and Ocimum gratissimum,*

I. Introduction

Ocimum gratissimum (OG) is a plant belonging to *Lamiceae* family; it is known as Africa basil/sweet b asil and in Nigeria as *efinrin*, *Nehonwu*, and *ai daya ta guda* by the Yoruba, Igbo and Hausa. It is, planted for its nutritional, medicinal and therapeutic effects. *O. gratissimum* is a plant with much potential and is useful in the treatment of many diseases; its antimicrobial [1], antidiarrhoeal [2], anti-inflammatory [3], antihypertensive [4], antidiabetic [5], hypolipidemic [6], hepatoprotective [7], antioxidant [8] and immunostimulatory effects [9] has been reported. Its major constituents include aromatic and volatile oil, linolenic acid, eugenol oil, thymol oil, ol eic acid, alkaloid, flavonoid, tannins, saponin and cardiac glycosides [10], which may play some roles in this ant ioxidative effect [11]. The antioxidant activity (AA) of *O. gratissimum* has been assessed using various assay te chniques such as lipid peroxidation assay [12] and reduced glutathione assay [13]. In this study we investigated t he effects of adding table salt (NaCl) and lime water (which is a common traditional practice) on the antidiabetic and antioxidant effects of Ocimum gratissimum in alloxan-induced diabetic rats.

II. Materials And Methods

2.1 Experimental Animals.

25 male Albino Wistar rats weighing 150-200g obtained from the Animal house, Achievers University, Owo were used for this study. The animals received a pellet diet and water *ad libitum*.

2.2 Drugs and chemicals.

Reduced glutathione (GSH), Thiobarbituric acid (TBA), Tricarboxylic acid (TCA), 5,5[1]- dithiobis[1]-2-n itrobenzene acid (DTNB) and Alloxan were obtained from Sigma (St, Louis, MO). Glucose oxidase, ALT, AST, Albumin and Total protein kits were obtained from Randox laboratory limited United Kingdom. Others were of analytical grades.

2.3 Induction of diabetes

Diabetes was induced by a double intraperitoneal injection of 100 mg/kg of alloxan monohydrate. Diab etes was confirmed by glucose oxidase method using a glucometer (accucheck) after 72 hours of alloxan injectio n. Rats with plasma glucose level ≥ 200 mg/dl were separated and used as diabetic in this study. Control rats rec eived distilled water.

2.4 Plant material

Ocimum gratissimum (*O.G*) plants were collected from Achievers University farm, Owo Ondo State, N igeria. The plant was identified and authenticated at the department of biological sciences of the University.

2.5 Preparation of *Ocimum gratissimum* extract.

The leaves of the plant were removed and air dried for 2 days. The dried leaves were grinded into powd er using a dry blender. 50 grams of the powdered leaves was extracted using 500mls of distilled water for 24 ho urs by cold extraction method. The filtrate was evaporated in a water bath at 60^{o}c to a constant weight as describ ed by Nwanjo and Uze, [12].The residual extract was used for further preparations. As follow;

Preparation of extract with water - 5 grams of the extract was dissolved in 100mls of distilled water.

Preparation of extract with NaCl- 5 grams of the extract was dissolved in 100mls of standard NaCl solution (1m ole/litre).

Preparation of extract with lime water-5 grams of the extract was dissolved in 100mls of standard 10% v/v lime water solution.

2.6 Experimental procedure

A total of 25 rats (20 treated diabetic rats and 5 control rats) were used in this experiment. The rats were divided into 5 groups of five rats each as follows

Group 1- Control untreated rats.

Group 2- Diabetic control rats,

Group 3- Diabetic rats receiving 5% O.G (400mg/kg body weight) in aqueous solution,

Group 4- Diabetic rats receiving 5% O.G (400mg/kg body weight) + NaCl and

Group 5-Diabetic rats receiving 5% O.G (400mg/kg body weight) + lime water,

The extracts were administered daily by feeding cannular for 6 weeks. At the end of 6 weeks all rats were sacrifi ced by humane method. Blood was collected in fluoride oxalate bottles for the estimation of plasma Glucose and EDTA bottles for estimation of plasma Albumin and Total protein. The liver of the rats were dissected out, was hed and homogenized with (1/10 w/v) of 50 mM Tris–HCl buffer (pH 7.4) and phosphate buffer pH 7.4 for MD A and GSH assays respectively.

2.7 Biochemical analysis

Plasma glucose was measured using enzymatic colorimetric diagnostic kits obtained from Randox Lab oratories, United Kingdom, in which the glucose oxidase/peroxidase/4-aminophenazone (GOD-PAP) method of Tietz [14] was employed. Absorbance was measured at 500 nm. The measurement of plasma albumin is based o n its quantitative binding to the indicator bromocresol green (BCG) method of Tietz [15] as applied in Randox k it was used for the determination of plasma Albumin. The Total Protein was estimated using biuret method as de scribed by Tietz [16].

2.7.1 Lipid peroxidation assay

Malondialdehyde (MDA) a product of lipid peroxidation in the liver was assayed using the method the method of Hunter *et al*., [17] modified by Gutteridge and Wilkins [18]. 0.2mls of tissue homogenate (Tris-HCl buffer pH7.5) was treated with 1ml each of Glacial acetic acid and 1% TBA, placed in a water bath at 37^{o}C for 1 5 min, cooled and centrifuged. The absorbance of the clear supernatant was measured against reference blank at 532nm.

2.7.2 Reduced Glutathione (GSH)

The level of GSH in the liver was determined by the method of Beutler *et al*., [19]. 1ml of 4% sulphosa licyclic and 1ml of the tissue homogenate (homogenised in 0.2 M phosphate buffer pH 7.3) was mixed and centr ifuged at 4,000 rpm for 5min. 0.5ml of the clear supernatant was added to 4.5 ml of Ellmans reagent (prepared by adding 40mg of 5,5^{1}- dithiobis^{1}-2-nitrobenzene acid (DTNB) in 100mlof 0.1M phosphate buffer. The absorb ance of the reaction was read at 412nm.

2.7.3 Liver AST and ALT

Aspartate Aminotransfarase (AST) and Alanine Aminotransferase (ALT), were estimated as described by Reitman *et al.,* [20] using diagnostic kits obtained from Randox Laboratories, United Kingdom.

2.8 Statistical analysis

All results were expressed as mean ± SEM. Data were analyzed by one-way analysis of variance (ANO VA), with statistical significant level at $p<0.05$. All analyses were performed using SPSS 17.0 package.

III. Results.

Table 1, shows the mean values of plasma glucose level, total protein and plasma albumin of control, di abetic, and diabetic treated rats. The results revealed that there was a significant ($p<0.05$) increase in fasting blo od glucose and decrease ($p<0.05$) in plasma protein in the diabetic control group when compared with the norma l control and the treated rats. A significant ($p<0.05$) decrease was observed in the plasma glucose of rats treated with O.G only when compared with those treated with O.G plus lime or NaCl. However the result revealed an in crease in the plasma Albumin and Total protein of those treated with O.G plus lime or NaCl when compared wit h those treated with O.G only. However there were no significant ($p<0.05$) differences in the plasma Albumin le vels of the experimental rats.

Table 2, revealed the results of the mean values of MDA (end-product of lipid peroxidation) and GSH i n the livers of experimental rats. The MDA levels in diabetic rat treated with *O.G* significantly ($p<0.05$) decreas ed when compared with the control. However a significant increased in the liver MDA level was observed in dia betic rats treated with the aqueous leaf extract of *O.G* plus lime water when compared with the controls. Conver sely a significant ($p<0.05$) decrease in the liver's GSH level in diabetic control rats when compared with the con trols was observed. While there was a significant ($p<0.05$) increase in liver GSH levels in rats treated with the a queous extract of *O.G.* The result also revealed a significantly ($p<0.05$) higher GSH levels in rat treated with *O. G* plus NaCl when compared with the control.

Table 3 showed the mean values of liver AST and ALT levels of the experimental rats. There was a sig nificant ($p<0.05$) decrease in the liver AST of diabetic rats compared with the control and the treated rats. Table 3 also revealed a significant ($p<0.05$) increase in the liver ALT level of rats treated with aqueous extract of *O.G* when compared with the control. However there was a significant ($p<0.05$) decrease in the liver ALT of rats tre ated with O.G plus NaCl when compared with the control and those treated with O.G only.

TABLE 1. The effects of adding NaCl or Lime water on aqueous leaf extract of *Ocimum gratissimum* on plasm a Glucose, plasma Albumin and Total protein of alloxan induced diabetic rats.

Groups	Plasma glucose(mg/dl)	Plasma Albumin(g/dl)	Total protein (g/dl)
Group 1	96.46±5.74	2.59± 0.23	1.52± 0.14
Group 2	310.62±40.50***	2.53 ±0.24	1.17 ± 0.06**
Group 3	191.27±10.00*	2.39 ± 0.02	1.33 ± 0.07
Group 4	245.82±36.26	2.81± 0.43	1.44 ± 0.06
Group 5	295.54±36.84	2.73 ± 0.19	1.49 ± 0.06

Values are expressed as mean ±SEM, P<0.05. *Significantly different from normal control and diabetic groups (p <0.05). **Significantly different from normal control and O.G treated diabetic rats (p<0.05). *** Significantl y different from normal control and treated diabetic groups (p<0.05).

TABLE 2. The effects of adding NaCl and Lime water on aqueous leaf extract of *Ocimum gratissimum,* on liver Malondialdehyde (MDA) and GSH of alloxan induced diabetic rats.

Groups	Liver MDA (µmMDA/g)	Liver GSH (µg/ml)
Group 1	14.45±5.45	5.00± 0.00
Group 2	15.10±8.79	2.33± 0.03*
Group 3	4.85±0.85***	13.5 ±1.50**
Group 4	13.7±7.04	8.66 ± 0.88
Group 5	23.5±7.86**	5.00± 1.73

Values are expressed as mean ±SEM, P<0.05. *Significantly different from normal control group and treated dia betic groups (p <0.05). ** Significantly different from normal control and other treated diabetic rats (p<0.05) ** *significantly different from normal control group, diabetic and treated diabetic groups (p <0.05).

TABLE 3. The effects of adding NaCl and Lime water on aqueous leaf extract of *Ocimum gratissimum,* on liver AST and ALT of alloxan induced diabetic rats.

Groups	Liver AST (U/I)	Liver ALT (U/I)
Group 1	53.00 ± 0.00	29.50± 0.50
Group 2	36.33 ± 2.60**	34.00±5.00
Group 3	47.00 ± 1.00	43.00± 3.00*
Group 4	44.33 ± 2.84	24.66± 0.80**
Group 5	43.00 ± 3.51	28.75 ± 4.50

Values are expressed as mean ±SEM, P<0.05. *Significantly different from normal control group (p <0.05) **significantly different from normal control and other treated diabetic rats (p<0.05)

IV. Discussion

Several studies have shown that antioxidants ameliorate a number of altered physiological and metaboli c parameters that occur as a result of type-2-diabetes [21, 22]. Also in clinical, experimental in vivo and in vitro

models of diabetes, antioxidant potential correlates with the degree of glycemic control and it decreases with pro longed diabetes [23]. The results of this study clearly indicated that the administration of aqueous leaf extract of *O. gratissimum* produced both hypoglycaemic, and antioxidant effect against Alloxan induced diabetes in rats. As shown in table 1, administration of aqueous extract of O.G, significantly reduced the plasma glucose levels o f diabetic rats in groups 3, 4 and 5 compared with the untreated diabetic rat. This is in correlation with the findin gs of [24]. However this hyperglycemic lowering effect was reduced in groups 4 and 5, this may be due to incre ased oxidative load by the NaCl or Lime water or may be due to the alteration of the chemical compositions of t he extract.

In this study the observed decrease in the concentration of plasma protein in diabetic rats may be due t o catabolism of proteins and nucleic acids which results in increase in the formation of non –protein nitrogenous compounds [25] However treatment of the diabetic rats with O.G significantly increased the concentration of pl asma protein and no significant difference was observed in plasma Albumin rats in groups 3, 4 and 5 when com pared with the control.

Reduced glutathione (gamma-glutamylcysteinylglycine (GSH)), a tripeptide with a free thiol group, is a major antioxidant in human tissues that provides reducing equivalents, for the glutathione peroxidase (GPx) cat alyzed reduction of hydrogen peroxide and lipohydroperoxides to water [13]. The decrease in the GSH level in d iabetic group represents increased utilization due to oxidative stress [26], while the significant increase in the G SH levels of the diabetic rats when treated with aqueous leaves extract of *O. gratissimum* as observed in table 2, is in support of the findings that Reduced glutathione (GSH) was preserved and enhanced by the oral administrat ion of Ocimum gratissimum in a previous study by [27]. However the addition of table salt and lime water reduc ed the antioxidant effects as observed in the significantly higher plasma glucose levels in rats treated with O.G p lus salt or Lime water, as well as a decrease in the GSH levels of rats in these groups (4 and 5) when compared with those in group 3. Addition of NaCl may result in increased plasma Na^+, while the addition of lime water ma y increase the plasma level of K^+ which ultimately leads to a decrease in urinary Na level. This increase may in turn lead to water retention thus contributing to elevated blood pressure which may lead to an imbalance in hom eostasis thus causing increase in oxidative stress or a reduction in the antioxidants GSH levels.

Diabetes represents a state of increased oxidative stress, which is mainly based on the evidence of increased lipi d peroxidation (LPO), or by indirect evidence of reduced antioxidant reserve, like superoxide dismutase (SOD) and catalase enzymes, in animal models [28]. Under normal physiological conditions, the concentrations of lipid peroxides in the tissues are low. Hu et al., [29] reported elevated levels of lipid peroxides in the plasma of diabe tic rats. Lipid peroxide-mediated tissue damage results in the development of both type I and type II diabetes. T he increase in liver MDA levels in diabetic control rats are in conformation with previous report documenting el evated serum lipid peroxide levels in diabetic subjects [30]. In this study, administration of aqueous extract of *O . gratissimum* significantly ($P< 0.05$) decrease the level of MDA in the tested liver, compared to diabetic rats (gr oup 2), indicating a decreased rate of lipid peroxidation. This may be due to decreased oxidative load. It may als o act by either directly scavenging the reactive oxygen metabolites, due to the presence of various antioxidant co mpounds [31] or by increasing the synthesis of antioxidant molecules. However the addition of NaCl and lime water to the extract of O.G significantly reduced this inhibitory effect, though the precise mechanism(s) and site (s) of action require further elucidation.

Increase in the hepatic enzymes levels, such as alanine aminotransferase (ALT), aspartate aminotranfer ase (AST) are good indicators of oxidative stress in several studies [32, 33], which also indicated possible dama ge of the affected tissues. Evaluation of liver alanine aminotransferase (ALT), aspartate aminotransferase (AST) in normal control, alloxan-induced diabetic and diabetic treated rats with O.G, O.G plus NaCl and O.G plus lim e water is revealed in table 3. The result revealed that AST enzymes levels decreased significantly in the liver of alloxan-diabetic rats (group 2) compared with normal control rats (group 1), and the O.G treated rats. A decrea se in the liver AST level is suggestive of hepatocellular damage, resulting from the leakage of hepatic enzymes i nto the blood stream. Treatment of diabetic rats with O.G significantly increased the activity of AST, with the aq ueous extract having higher effect when compared with the extracts mixed with NaCl and lime water. There was no significant difference in the levels of liver ALT in groups 1, 2, 4 and 5, however treatment of diabetic rats wi th O.G only significantly increased the activity of ALT in group 3 when compared with others.This result indica ted that *O. gratissimum can* inhibits or prevent oxidative stress/damage that could occur during normal metaboli c processes. While the addition of lime water to the extract, may be responsible for the reduced liver AST and A ST observed in group 4 which is suggestive of hepatocellular damage.

V. Conclusion

It could be concluded that Ocimum gratissimum may reduce hyperglycaemia and MDA levels in diabet es while increasing GSH level which is one of the body's major antioxidant defence system. However the additi on of NaCl (table salt) or lime water (which is a common traditional practice) may inhibit these activities. Furthe r in vitro and in vivo studies should be carried out to elucidate the precise inhibitory mechanisms of action.

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