Journal of Scientific Research & Reports
2(1): 408-422, 2013; Article no. JSRR.2013.028

SCIENCEDOMAIN *international*
www.sciencedomain.org

Anti-Gastric Ulcerative Activity of *Ocimum gratissimum* in Streptozotocin-Induced Diabetic Rats

Okon Uduak Akpan[1*], Obembe Agona Odeh[2], Olubobokun Titilope Helen[1] and Owu Daniel Udofia[2]

[1]Department of Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Uyo, Uyo, Akwa Ibom State, Nigeria.
[2]Department of Physiology, College of Medical Sciences, University of Calabar, Calabar, Cross River State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author OUA designed the study, coordinated the research, and wrote the first draft of the manuscript. Author OAO managed the analysis and interpretation of data. Author OTH wrote the protocol and managed the literature searches. Author ODU supervised and guided the entire experimental procedure. All authors read and approved the final manuscript.

Research Article

Received 30th March 2013
Accepted 13th June 2013
Published 28th June 2013

ABSTRACT

Aim: It has been observed that Diabetes Mellitus (DM) cause altered acid secretion and increased rate of ulceration. *Ocimum gratissimum* (OG) have been reported to possess hypoglycemic properties. This study therefore set out to determine the effect of DM on ulcerogenic indices and how OG could ameliorate them.
Methodology: The phytoconstituents and median lethal dose of the plant extract was determined before administration. Eighteen rats were used; the animals were divided into three groups of six rats each. Group 1 was the control and were given normal feed only. Group 2 was diabetic untreated rats (DM) while group 3 was OG treated diabetic rats (DMT). All the groups had access to water ad libitum. After 28 days, the gastric acid output, mucus secretion rate and ulcer scores were determined.
Results: The result showed that the basal acid output in the DM group was significantly higher than control. The peak acid output in the DMT was significantly lower (P=.001)

**Corresponding author: E-mail: chairmo2013@gmail.com;*

when compared with control and DM. The mean mucus secretion was significantly (p=.001) lower in the DM and DMT compared with control. The mucus secretion in DMT was significantly (p=.001) higher compared with DM. The mean ulcer score was significantly higher in DM than in control (p =.01) and significantly higher in DMT than in control (P =.01) but significantly lower than in DM group (P =.01).
Conclusion: We therefore conclude that OG mitigate ulcerogenic activities in STZ - induced diabetic rats by reducing gastric acid secretion and increasing mucus secretion.

Keywords: Ocimum gratissimum; diabetes mellitus; gastric ulcer; Gastric acid and mucus secretion.

1. INTRODUCTION

The pathophysiology of altered acid secretion in DM has been investigated extensively in rodent models. Since gastric acid secretion is dependent on the integrity of the vagal nerves, chemo and mechanoreceptors, neuropeptides and hormones, an effect of diabetes is to be expected. Streptozotocin (STZ) induced diabetic rats most often show increased gastric acid secretion [1,2] and increased rate of ulceration [3,4]. This effect is exacerbated by fasting and is reversed by hypoglycemia [5] but not by insulin replacement [6]. It thus appears that insulin lack is not the ulcerogenic stimulus, and raises the possibility that absence of gastric–inhibiting factors (e.g. amylin, PYY, GLP – 1), which may be absent or reduced in DM, could be implicated. In contrast to the majority of finding of acid hypersecretion, one study noted no difference between diabetic and non-diabetic animals in pentagastrin-stimulated acid secretion [7], and one study described a reduction in acid secretion in alloxan-treated rats [8]. A direct toxic effect of STZ on gastric mucosa has been proposed as a mechanism of increased ulceration in STZ - induced diabetes. The constancy of findings of acid hypersecretion and ulceration in insulinopenic diabetes invoked by diverse insults (Chemical and autoimmune) indicates that this GI disturbance is a direct consequences of DM, and perhaps of β – cell deficiency.

Central control of gastric acid secretion is mediated via a cholinergic pathway that includes the nucleus tractus solitarius (NTS), area postrema. (AP), dorsal motor nucleus of the vagus [9,10] and capsaicin- sensitive vagal afferents [11]. Insulin stimulation of gastric acid secretion [12] appears to depend upon its hypoglycaemic effects. Increasing plasma glucose concentration inhibits gastric acid secretion [13,14], including that simulated by insulin [15] and amino acids [16]. In contrast, glucagon-induced inhibition of gastric acid secretion appears to be independent of its effect on plasma glucose [17]. Amylin, which has a high density of receptors in the AP/NTS [18], is a potent inhibitor of gastric acid secretion [19], independent of changes in plasma glucose [20] and prevent gastric erosion in response to a number of irritant [21,22].

Ocimum gratissimum (OG) – Lamiaceas commonly known as ‘scent’ leave, has been used naturally in the treatment of different diseases [23,24,25]. OG is believed to originate from central Africa and tropical Asia. It is also found in West Africa. In Nigeria it is found in the Savannah and Coastal areas. The wide usage of OG is as a result of its vague biological importance, ranging from traditional, nutritional and medicinal values. It is used as ‘seasoning’ leave due to its peculiar aroma and as vegetable.

Anti-ulcerogenic pharmacological effect of various plants can be traced to their flavoniods content. Flavoniods are “diphenylpropanes” that occur in plants, as more than 400 flavoniods

have been found and are frequent in human diet [26].Flavoniods have been found to have membrane stabilizing properties and also affect some process of intermediary metabolism and inhibit lipid peroxidation in different systems [26]. Some have been shown to increase mucosal content of prostaglandins and mucus in gastric mucosa, showing cytoprotective effect. The active compounds of plant: flavoniods, terpenes and tannins may be regarded as possible active compounds against gastric lesions by acting as protective factor or increasing antioxidant activity.

The major aim of this study was therefore to determine the effect of DM on ulcerogenic indices and how OG reported for its anti-ulcerogenic activity can alter these effects.

2. MATERIALS AND METHODS

2.1 Plant Materials and Preparation of Aqueous Extract

The leaves of *Ocimum gratissimum* were obtained from the University of Calabar Botanical Garden and identified by the Chief Herbarium Officer of Botany Department of University of Calabar. The fresh leaves were rinsed with water to remove sand and debris and then allowed to drip off water. The leaves were then dried under shade for two days and then transferred into AstellHearson Oven and dried at a temperature range of 40 – 45ºC.

The dried leaves were then ground in an electric blender into fine powder to give a gram weight of 527grams. This 527g weight was soaked in 2.65 liters of water (distilled water) and allowed over night for about 15 hours and stirred at interval. The mixture was filtered using a satin mesh material and the final filtrate was gotten by using Whatman's filter paper size 1. The final filtrate was dried in the Astell Hearson Oven at 45ºC to obtain a brown gummy paste. A mettler P163 electronic weighing balance was used to weigh the gummy paste before stock solution was prepared. The stock solution of the extract was prepared by dissolving 15gm of extract in 10ml of water to give a concentration of 1500mg/ml. The stock solution was labeled appropriately and refrigerated at 4ºC until required for use. The median lethal dose (LD_{50}) of the plant extract was determined by method of Lorke [27].

2.1.1 Determination of phytoconstituents

The phytoconstituents of the extracts was determined and were screened for the presence of carbohydrates, tannins, alkaloids, saponins, phenolics, anthraquinones and cardiac glycosides as described by Trease and Evans [28] and Sofowora [29]

2.2 Animals Preparation, Experimental Groupings and Treatment

Eighteen rats were used for the study, the animals were divided into three groups and were assigned randomly into each group which was made up of Six (6) rats each and housed in cages assigned to them.

The first group was assigned as the control and were fed with normal rat chow (feed) orally. The second group contained streptozotocin induced diabetic rat which were left untreated. The third group of animals contained the test group which were streptozotocin induced diabetic rats treated with aqueous leaf extract of *Ocimum gratissimum*. All the animal groups were allowed access to water ad libitum. The experimental procedures involving the animals

and their care were in line with the approved guidelines by the local research and ethical committee.

2.2.1 Induction of diabetes mellitus

Diabetes mellitus was induced by a single injection of 65mg/kg streptozotocin. The injection was given intraperitoneally. The state of diabetes was observed after 48 hours by the symptoms of polyuria and glucosuria and this state was confirmed using uristic test strip (Bayer Health Care LLC, USA). Also, the blood glucose level was tested 1 week after induction using a Glucometer (ACCU-CHECK Advantage II, Roche Diagnostics (GmbH, Germany) and ACCU-CHECK Advantage II test strips.

2.2.2 Extract administration and observation

One week after induction of diabetes, the extract was administered per oral to the DMT group at a dose of 1500 mg/kg body weight once daily for 28 days. Administration was facilitated by the use of a syringe and Orogastic tube.

2.3 Measurement of Gastric Acid

Measurement of gastric acid was done by the continuous perfusion method of Gosh and Schild [30] as modified by Osim et al. [31]. Rats from the control, DM and DMT groups were fasted 24 hour before the start of experiments. 6ml/kg of 25 per cent (v/v) solution of urethane (Sigman, UK) was given intra-peritonally to anaesthetize each rat. The trachea was exposed and cannulated. Another cannula was passed through the mouth and esophagus until it reached the stomach. It was then tied firmly in place with a ligature around the oesophagus in the neck. The abdomen was then opened along the linea alba to minimize bleeding. The stomach was exposed and the pyloric end cannulated at its junction with the duodenum. Isotonic (0.9 per cent) saline was introduced gently via the esophageal cannula to wash out any stomach content. The perfusate was allowed to flow freely after clearing the food particles. The abdominal incision was then covered with a moist cotton wool dipped in normal saline. The stomach was continuously perfused with normal saline at the rate of 1ml/min.

The pH of the saline was maintained at 7.0 and the body temperature of the rat was maintained at 37ºC by a heating lamp and a rectal thermometer was inserted in the rat to monitor its body temperature. The flow was adjusted to give an effluent volume of about 1ml per minute. The effluent was collected at 10 minute intervals and care was taken not to ligate the blood vessels as this may lead to stained perfusate. Each 10 minute perfusate after adding two drops of phenolphthalein as indicator was titrated against 0.01N NaOH (May and Baker UK) to determine its total acidity. The experiments were repeated using histamine as acid secretagogue. The dose of the histamine was 100 mg/Kg body weight administered subcutaneously (Sc). Gastric acid output in the effluent sample was measured by titrimeric analysis.

2.4 Gastric Ulceration

The animals were starved for 24 hours. Under anaesthesia (6ml/1kg of 25 per cent v/v solution of urethane), a pyloric incision was made and a cannula inserted and kept in place by tying with a tread. The stomach of the animals was instilled with 1.5 ml of acid alcohol,

prepared from equivolume of 0.1N HCl and 70 per cent methanol [32]. The instillation was via the pyloric incision. The animal was left to stay for an hour. Then, the stomach was isolated, washed and cut open along the greater curvature and rinsed with normal saline. Pins were used to fasten the tissue in place for proper visualization. A magnifying lens and a vernier caliper were used to measure the extent of ulceration. Scoring of ulcer spots was by the method of Alphin and Wards [33] and Adeniyi and Olowokorun [32]. Digital photographs were taken. Representative specimens are shown in plates 1, 2 and 3.

2.5 Statistical Analysis

All results are presented as mean + standard error of mean. Three sets of data were analyzed using one way ANOVA, followed by the least significant difference (LSD) procedure for significant F values, (P=.05) was considered significant. Computer software SPSS and Excel Analyzer was used for the analysis.

3. RESULTS AND DISCUSSION

3.1 Comparison of Basal Acid Secretion in Control, DM and DMT Groups of Rat

Mean basal acid secretion in control and diabetic treated (DMT) rats was 4.00 ±0.00µmol/10mins and 3.63 ± 0.15µmol/10mins respectively while the diabetic untreated (DM) rats had mean basal acid output of 4.60±0.09µmol/10mins. The result showed that diabetic untreated rats had a basal acid output which was significantly higher than that in control (P=.001) and diabetic treated (P=.05) (Fig. 1)

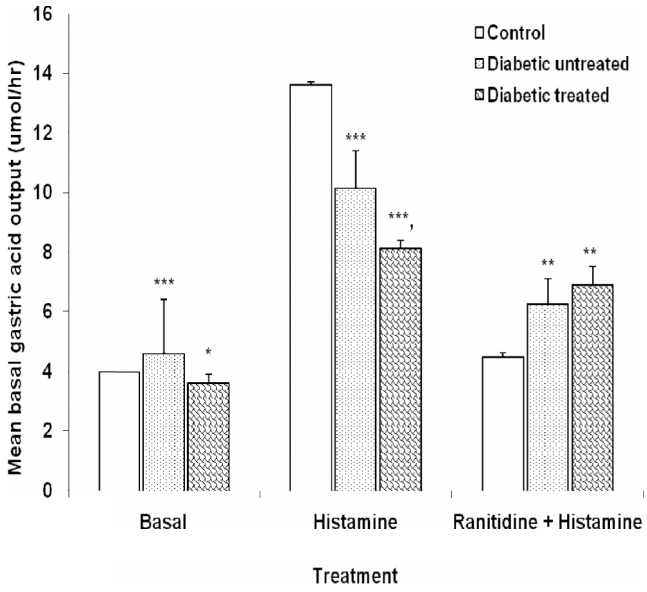

Fig. 1. Comparison of mean acid output and effect of histamine, ranitidine + histamine on gastric acid secretion in the different experimental groups

*Test drugs: significant from normal control, ***p=.001, *p=.05 vs control; c = p=.001 vs diabetic untreated.*

Mean ± S.E.M = Mean values ± Standard error of means of six experiments

3.2 Comparison of Peak Acid Output Following Histamine Administration in Control, DM and DMT Groups of Rats

The peak acid output in diabetic untreated rats (14.00 ± 1.53 µmol/10mins was significantly lower (P=.001) than that in control rats 19.20 ± 2.31µmol/10mins. However, the peak acid output in the diabetic treated rats 4.00 ± 0.25µmol/10mins was significantly lower (P=.001) when compared with control or diabetic untreated group, Fig. 2.

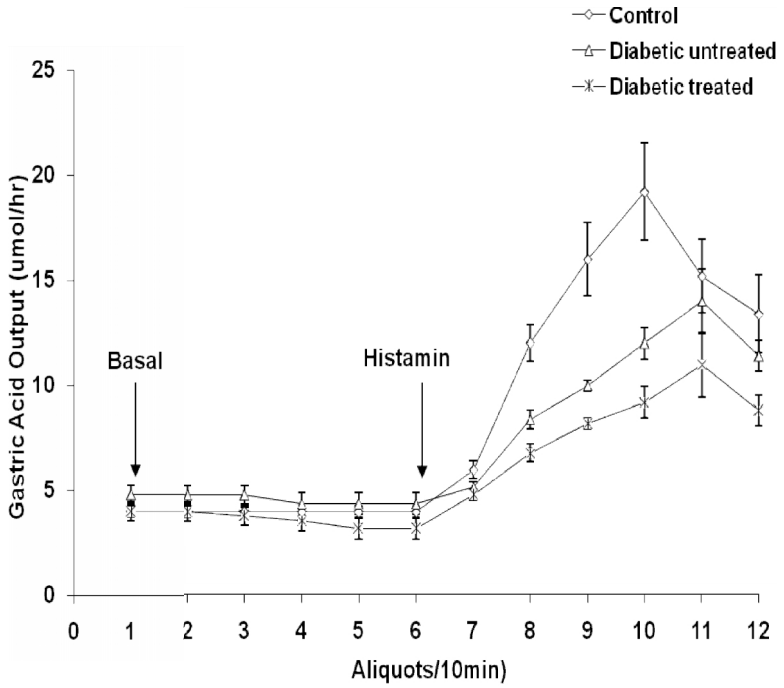

Fig. 2. Comparison of effect of subcutaneous administration of histamine on gastric acid secretion in different experimental groups

Mean ± S.E.M = Mean values ± Standard error of means of six experiments.

3.3 Comparison of Effect of Ranitidine on Histamine Stimulated Acid Secretion in Control, DM and DMT Groups Of Rats

Histamine stimulated peak acid output in control, DM and DMT was significantly reduced after administration of histamine-H_2 blocker, ranitidine when compared to histamine stimulated peak acid output in the three groups without the blocker, ranitidine. These reductions in the PAO following ranitidine administration were greater in the control than in the test groups, Fig. 3

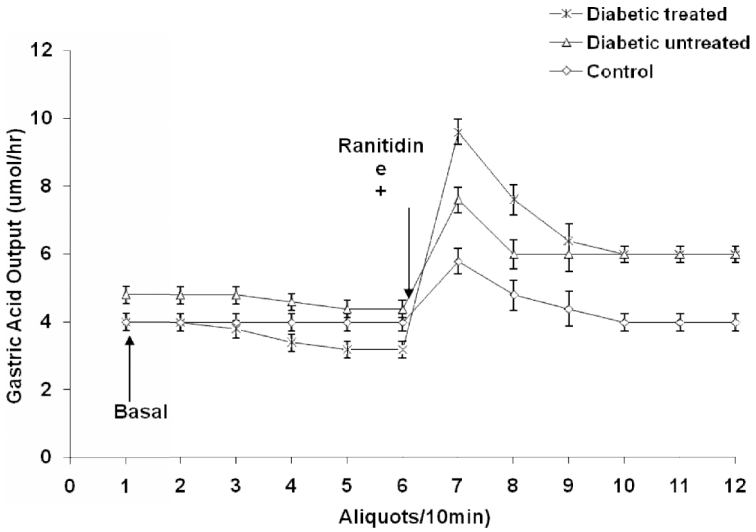

Fig. 3. Comparison of effect of subcutaneous administration of ranitidine + histamine on gastric acid secretion in different experimental groups

Mean ± S.E.M = Mean values ± Standard error of means of six experiments.

3.4 Mucus Secretion (Weight)

The mean mucus secretion in the control, diabetic untreated and diabetic treated rats were 1.17 ± 0.01, 0.46 ± 0.06 and 0.89 ± 0.03g respectively. The mean mucus secretion was significantly (p=.001) lower in the diabetic untreated and diabetic treated groups compared with control. The mucus secretion in diabetic treated group was significantly (p=.001) higher compared with diabetic untreated group, Fig. 4.

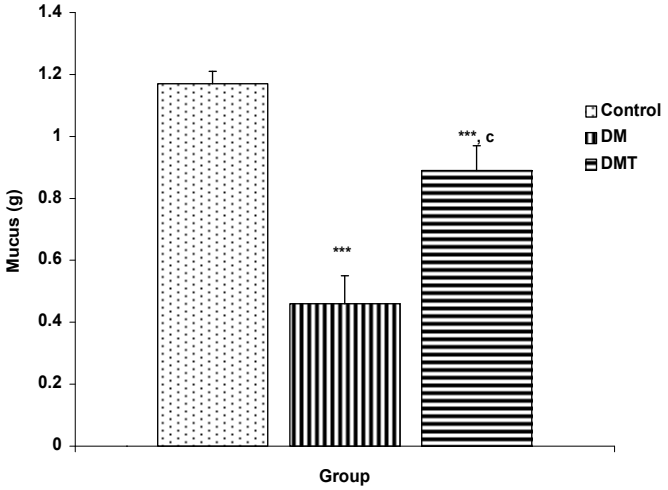

Fig. 4. Comparison of mean mucus concentration in the different experimental groups

*Test drugs: significant from normal control, ***P=.001 vs control, c = P=.001 vs diabetic-untreated. Mean ± S.E.M = Mean values ± Standard error of means of six experiments*

3.5 Comparison of Ethanol – Induced Ulcer in the Different Experimental Groups

The mean ulcer score for control rats was 7.3 ± 0.40, diabetic untreated rats was 18.1 ± 1.46 and for diabetic treated rats 14.3 ± 0.4. The result showed that the mean ulcer score was significantly higher in diabetic untreated than in control rats (p=.01). The mean ulcer score was significantly higher in diabetic treated rats than in control (P=.01) but lower than the diabetic untreated group (P=.01), Fig. 5.

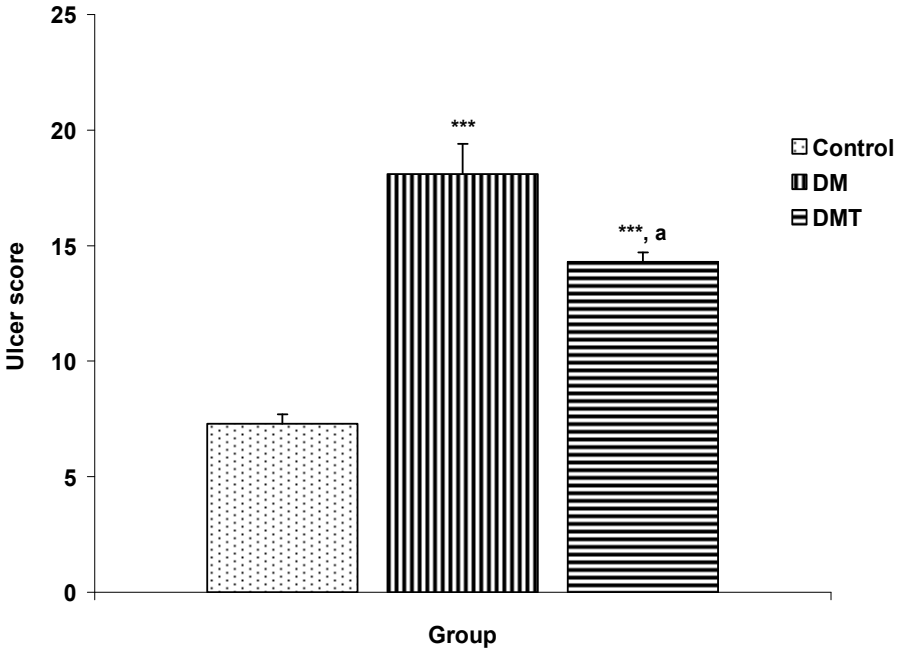

Fig. 5. Comparison of mean ulcer score in the different experimental groups
*Test drugs: significant from normal control, ***P=.001 vs control, a = P=.05 vs diabetic-untreated. Mean ± S.E.M = Mean values ± Standard error of means of six experiments*

3.6 Fasting Blood Glucose in the Different Experimental Groups of Rats

The mean values of fasting blood glucose in the control, DM and DMT experimental groups were 2.46 ± 0.192, 28.2 ± 1.52 and 16.5 ± 1.21mmol/l for control, DM and DMT groups respectively. All the groups were significantly different. The DM and DMT groups were significantly higher ($p < 0.001$) than the control. The DMT group was signifi-cantly lower ($p < 0.01$) than the DM group. (Fig. 6)

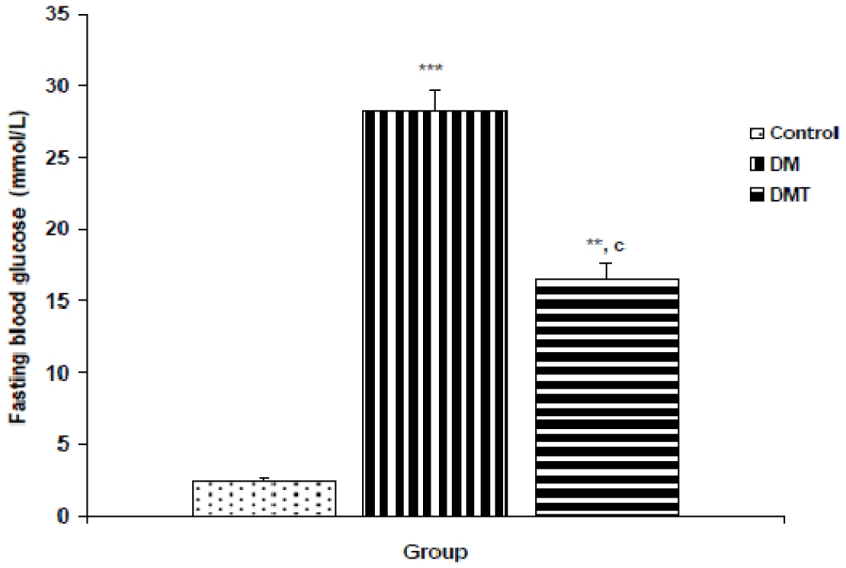

Fig. 6. Fasting blood glucose in the control, DM and DMT experimental groups of rat
*Test drugs: ***P<0.001, **P<0.01 vs control; c = P<0.001 vs DM*
Mean ± S.E.M = Mean values ± Standard error of means of six experiments

3.7 Histological Pictures of Ulcer Score in Different Experimental Groups

Plate 1. Ulcer scores for control group

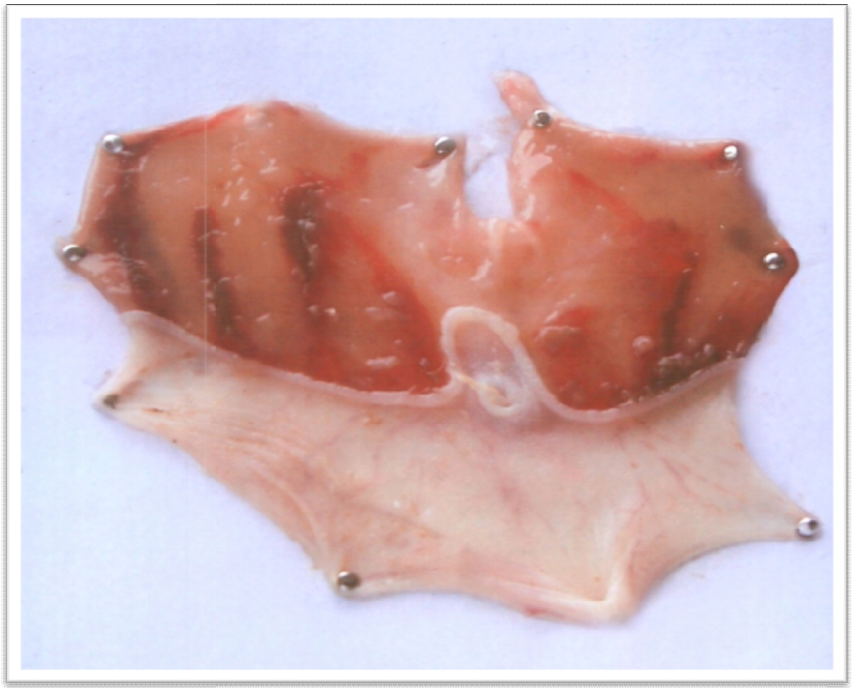

Plate 2. Ulcer scores for diabetic untreated group

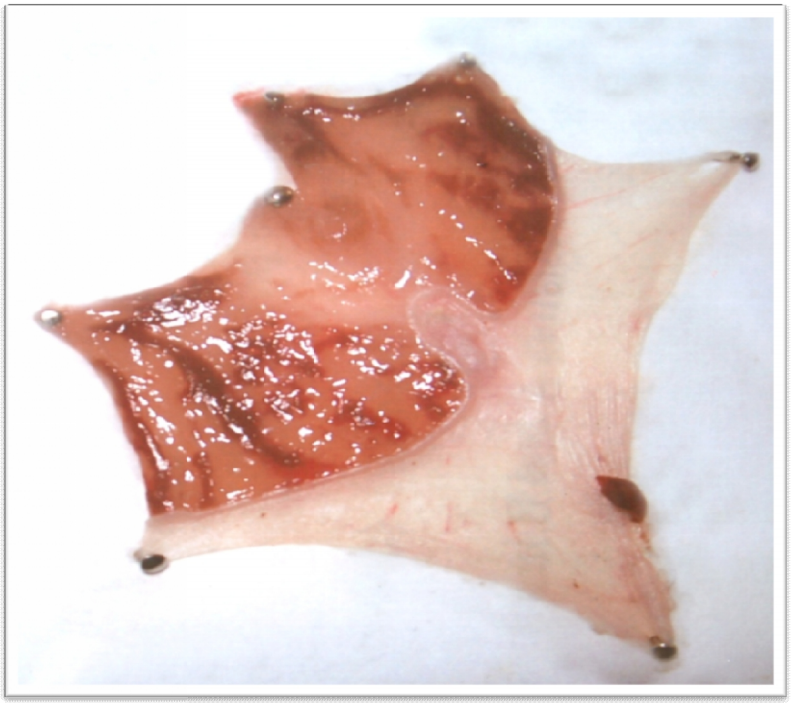

Plate 3. Ulcer score for diabetic treated group

3.8 Discussion

The result from this study has confirmed the earlier reported hypoglycemic effect of *Ocimum gratissimum* (OG). The blood glucose level in the DMT group was significantly lower than the DM group. In the DM and DMT groups, the blood glucose level were significantly higher than the control group, thus confirming the induction of DM by streptozotocin administration. The blood sugar level in the DMT group did not return to the normal level but was significantly lower (i.e. near normal) compared to the DM group.

The highest basal gastric acid output was obtained in the DM group, followed by the control while the DMT group had the least. As noted earlier, since gastric acid secretion is dependent on the integrity of the vagal nerves, chemo – and mechanoreceptors, neuropeptides and hormones, an effect of diabetes is to be expected. In so – called early diabetes, peak acid output was reported to be normal after histamine and pentagastrin administration, but the response to sham feeding or insulin – induced hypoglycemia was reduced [34,35]. These observations suggest that vagal impairment may be responsible for a decreased peak acid output. However, it should be recognized that impairment of the gastric acid secretory responses to sham feeding and other stimuli may potentially be due to hyperglycemia [36] rather than irreversible vagal neuropathy. The outcomes of rodent studies that have evaluated the response to direct stimulation of parietal cells have been conflicting. In some studies the acid secretory response to histamine was suppressed, while others reported the opposite [8,37] possibly due to the duration of diabetes. Our study showed peaked levels of gastric acid output in the control group followed by the DM group and the DMT group still with the lowest, after histamine administration. However, the reduced gastric acid secretion in the DMT group appears to be associated with the effect of OG on lipid peroxidation. The influence of lipid peroxidation on plasma thyroxine (T_4) and triodothyronine (T_3) which enhances $Na^+ – K^+$ ATPase activity in cells has been reported; the enhancement of this enzyme increases a number of pump units which may likely increase acid secretions [38]. Flavonoids, a major phytochemical constituent of OG have been reported to inhibit lipid peroxidation in different systems [26]. Therefore, the reduction in gastric acid output observed in the DMT group maybe related to the above activity of OG. The DM group was prone to peptic ulcer due to increase basal gastric acid output. The DMT group had decrease basal gastric acid output meaning that OG may be beneficial in preventing peptic ulcer disease. Peak acid output following histamine administration was lower in DM and DMT groups, suggesting impairment of histamine H_2 receptors.

A major role for histamine in physiological regulatory mechanism of gastric acid secretion was established with the advent of the histamine H_2- receptor antagonists, which have *in vivo* been shown to inhibit virtually all forms of basal and stimulated secretion [39]. Potentiating interactions between histamine and the other secretory stimulants at the parietal cell level have led to the hypothesis that histamine acts in a permissive role, markedly amplifying the effect of gastrin and acetylcholine on parietal cell receptors [40]. It is the removal of this permissive effect of histamine which accounts for the inhibitory actions of H_2-receptor antagonists such as ranitidine that was used in this study.

From our result therefore, OG appears to act as histamine H_2-antagonists to inhibit gastric acid secretion. It is also possible that OG might have blocked the activity of gastrin in stimulating the histamine forming enzyme, “histidine decarboxylase”. The antioxidants phytoconstituents of OG could also contribute to its anti-ulcerative properties probably acting through activation of non enzymatic and enzymatic antioxidants and some related growth factors.

The adherent mucus mass was heaviest in the control group followed by the DMT group and, the DM group was the least. This may be one of the causes of the high incidence in peptic ulcer score obtained in DM in this study. Since mucus protects the mucosa against high acidity and mucus secretion was the least in the DM group, there was a higher incidence of ulcer score in the DM group. This may also explain the high basal acid output in the DM group since mucus entraps bicarbonate ions that neutralize gastric acid [31].

Therefore, reduced mucus secretion will favour high basal gastric acidity as was the case in the DM group. Alanko et al. [26] had reported that the flavonoid constituent of OG have membrane stabilizing properties and also increase mucosal content of prostaglandins. The latter enhances mucus production from the gastric mucosa [41]. This may then explain the higher mucus secretion in the OG treated group when compared to the DM group.

Different mechanisms are involved in the formation of gastric mucosal lesions. Gastric ulceration occurs when there is an imbalance between protective and aggressive factors. The highest ulcer score was recorded in the DM groups, seconded by the DMT group. The control group had the lowest score. From this result it is possible that diabetes is a risk factor in the development of gastric ulcer disease. Helicobacter pylori infection is currently regarded as the main causative factor for gastritis and is responsible for the vast majority of peptic ulcer [42]. However, studies investigating the association of H. pylori infection with diabetes have yielded inconsistent results, with a substantial variation in the reported prevalence from 30 percent to 80 percent [43,44,45]. However, this study did not investigate the presence of H. pylori. The high basal acid output and low mucus secretion are the likely factors contributing to the higher incidence of ulcer scores in the DM group.

4. CONCLUSION

The results from this study has demonstrated the ulcerogenic effect of DM and has given indication that treatment with OG mitigate this diabetic complication by reducing gastric acid secretion and increasing gastric mucus secretion.

ACKNOWLEDGEMENTS

We hereby acknowledge the support of Dr Inyene E. Umoren MD, Bay Pines Veterans Afffairs, Medical center, USA and contribution of the Laboratory Staff of Physiology Department and Staff of the Histopathology Department of the University of Calabar, Cross River state, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Lin CY, Yeh GH, Hsu FC. Gastric acid secretion in streptozotocin-diabetic female rats. Clinical Journal of Physiology. 1991;34:179-186.
2. Piyachaturawat P, Poprasit J, Glinsukon T, Wanichanon C. Gastric mucosal lesions in streptozotocin-diabetic rats. Cell Biology International Report. 1988;12:53-63.
3. Goldin E, Ardite E, Elizalde JI. Gastric mucosal damage in experimental diabetes in rats: role of endogenous glutathione. Gastroenterology. 1997;112:855-863.

4. Chabre O, Liakos P, Vivier J. Gastric inhibitory polypeptide (GIP) stimulators cortisol secretion, cAMP production and DNA synthesis in an adrenal adenoma responsible for food dependent Cushing's syndrome. Endocrinology Research. 1998;24:851-856.
5. Arai I, Hirose-Kijima H, Usuki-Ito C, Muramatsu M, Otomo S. Putative role of endogenous insulin in cystamine-induced hypersecretion of gastric acid in rats. European Journal of Pharmacology. 1991;202:213-219.
6. Piyachaturawat P, Poprasit J, Glinsukon T. Gastric mucosal secretions and lesions by different doses of streptozotocin in rats. Toxicology Letters. 1991;55:21-29.
7. Baydoun R, Dunbar JC. Impaired insulin but normal pentagastrin effect on gastric acid secretion in diabetic rats: a role for nitric oxide. Diabetes Research & Clinical Practice 1997;38:1-8.
8. Ozeelikay AT, Altan VM, Yildizoglu-Ari N, Altinkurt O. Basal and histamine-induced gastric acid secretion in alloxan diabetic rats. General Pharmacology. 1993;24:121-126.
9. Gedulin B, Jodka C, Hoyt J, Young A. Evidence for amylin resistance for inhibition of gastric emptying in hyperamylinemic Fatty Zucker rats. Endocrine Society 81st Annual Meeting Program and Abstracts. 1999:217.
10. Silvestre RA, Rodriguez-Gallardo J, Gutierrez E, Garcia P. Failure of amylin to directly affect glucagon release. Study in the perfused rat pancreas. Diabetologia. 1999;42:A146.
11. Sakaguchi T, Sato Y. D-Glucose anomers in the nucleus of the tractus solitarius can reduce gastric acid secretion of rats. Experimental Neurology. 1987;95:525-529.
12. Isenberg JI, Stening GF, Ward S, Grossman, MI. Relation of gastric secretory response in man to dose of insulin. Gastroenterology. 1969;57:395-398.
13. Moore JG. The relationship of gastric acid secretion to plasma glucose in five men. Scandinevian Journal Gastroenterology. 1980;15:625-632.
14. Lam WF, Gielkens HAJ, Coenraad M, Lamers CBHW, Masclee AAM. Effect of insulin on basal and cholecystokinin stimulated exocrine pancreatic secretion in humans. Pancreas. 1999;18:252-258.
15. Stacher G, Shernthaner G, Francesconi M, Kopp HP. Cisapride versus placebo for 8 weeks on glycaemic control and gastric emptying in insulin-dependent diabetes: a double-blind cross-over trial. Journal of Clinical Endocrinology and Metabolism. 1999;84:2357-2362.
16. Lam WF, Masclee AA, Muller ES, Lamers CB. Effect of hyperglycemia on gastric acid secretion and gastrin release induced by intravenous amino acids. American Journal Clinical Nutrition. 1995;61:1268-1272.
17. Loud FB, Holst JJ, Rehfeld JF, Christiansen J. Inhibition of gastric acid secretion in humans by glucagon during euglycemia, hyperglycemia, and hypoglycemia. Digestive Disease of Science. 1988;33:530-534.
18. Zaidi M, Pazianas M, Shankar VS. Osteoclast function and its control. Experimental Physiology. 1993;78:721-739.
19. Hirsch IB. Type 1 diabetes mellitus and the use of flexible insulin regiments. American Family Physician. 1999;60:2343-2352, 2355-2356.
20. Gedulin BR, Lawler RL, Jodka CM, Young AA. Comparison of effects of amylin, glucagon-like peptide-1 and exendin-4 to inhibit pentagastrin-stimulated gastric acid secretion. Diabetologia. 1997;40(suppl 1):A300.
21. Limb C, Tamborlane WV, Sherwin RS, Pederson R, Caprio S. Acute incretin response to oral glucose is associated with stimulation of gastric inhibitory polypeptide, not glucagon-like peptide in young subjects. Pediatric Research. 1997;41:364-367.

22. Guidobono F, Pagani F, Ticozzi C, Sibilia V, Netti C. Investigation on the mechanisms involved in the central protective effect of amylin on gastric ulcers in rats. British Journal of Pharmacology. 1998;125:23-28.
23. Onajobi FD. Smooth muscle contracting lipid soluble principles in chromatographic fractions of *Ocimum gratissimum.* Journal of Ethnopharmacology. 1986; 18: 3-11.
24. Ilori M, Sheteolu AO, Omonibggehin EA, Adeneye AA. Antidiarrhoeal activities of *Ocimum gratissimum (*Lamiaceae). Journal of Diarrhoeal Diseases Research. 1996;14:283-285.
25. Orafidiya OO, Elujoba AA, Iwalewa FO, Okeke IN. Evaluation of antidiarrhoeal properties of *Ocimum gratissimum* volatile oil and its activity against enteroagregative *Eschrichia coli*. Pharmacology Letters. 2000; 10: 9-12.
26. Alanko J, Riuffa A, Holm P, Mulda I, Vapatalo H, Metsa-Ketela T. Modulation of Arachidonic acid Metabolism by plants: Relation to their structure and antioxidant /per-oxidant properties. Free Radical Biology and Medicine. 1999;28(suppl 1-2):193-201.
27. Lorke D. A new approach to practical acute toxicity testing. Arch. Toxicol. 1983;54:275-287.
28. Trease GE, Evans WC. Trease and Evans' Pharmacognosy: A Physician's Guide to Herbal Medicine. 1984; 13th Edition, Bailliere Tindall London.
29. Sofowora LA. Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd, Ibadan. 1984;85-82.
30. Gosh MN, Schild HD. Continuous recording of gastric acid secretion in rats. British Journal of Pharmacology. 1958;13:54-61.
31. Osim EE, Nneli RO, Efem SE, Etta KM. The effect of oral administration of aqueous extract of plantain (*musa peradisca)* on gastric acid secretion in albino rats. Nigeria journal of Physiological science. 1991;7(1): 22-28.
32. Adeniyi KO, Olowookorun MO. Intestinal fluids and glucose transport in rats. Effects of thyroidectomy and thyroxine administration. Nigeria Journal of Physiological Sciences. 1987;3:61-66.
33. Alpin RS, Ward JW. Action of hexapyronium bromide on gastric secretion in dogs and ulceration in rats. Archives international De Pharmacodyn Therapeutique. 1967;167:82-100.
34. Langer L. Pentagastrin - and insulin-induced secretion in diabetes mellitus. Acta Medica Scandinavica. 1972;191:471-475.
35. Feldman M, Corbett DB, Ramsey EJ, Walsh JH, Richardson CT. Abnormal gastric functions in longstanding, insulin-dependent diabetic patients. Gastroenterology. 1979;77:12-17.
36. Scarpello JH, Hague RV, Cullen DR, Sladen GE. The ^{14}C-glycocholate test in diabetic diarrhea. British Medical Journal. 1976; 2:673-675.
37. Tashima K, Nishijima M, Fujita A, Kubomi M, Takeuchi K. Acid secretory changes in streptozotocin-diabetic rats: different responses to various secretagogues. Digestive Disease Sciences. 2000;45:1352-1358.
38. Dauncey MJ, Ramsden DB, Kapadi AJ, Macari M, Ingram DL. Increase in plasma concentration of 3, 5, 3-triiodothryronine and thyronine after a meal and its dependence on energy intake. Hormonal Metabolism Research. 1983;15(10):499-500.
39. Parsons ME. Some speculations on the physiological control of gastric secretion. In: Torsoli A, Lucchelli PE, Brimblecombe RW, eds. H_2-antagonists. European Symposium. Capri. Amsterdam: Excerpta Medica. 1980:243-50.
40. Parsons ME. Histamine and the pathogenesis of duodenal ulcer disease. Gut 1985;26:1159-1164.
41. Fox SI. The Digestive System. Human Physiology (8th Edition) MC Graw Hill. 2008;560–591.

42. Edwards CRW, Bouchier IAD, Haslett C, Chilvers EE. Diabetes Melittus in *Davidson's* Principle and Practice of Medicine (10th Edition) Churchhill Livingstone, London. 2008;724–774.
43. Gasbarrini A, Ojetti V, Pitocco D, De Luca A. *Helicobacter pylori* infection in patients affected by insulin-dependent diabetes mellitas. European Journal of Gastroenterology & Hepatology. 1998;10:469-472.
44. Quatrini M, Boarino V, Ghidoni A, Baldassarri AR. *Helicobacter pylori* prevalence in patients with diabetes and its relationship to dyspeptic symptoms. Journal of Clinical Gastroenterology. 2001;32:215-217.
45. Xia HH, Talley NJ, Kam EP, Young LJ. *Helicobacter pylori* infection is not increased or linked to upper gastrointestinal symptoms in diabetes mellitus. American Journal of Gastroenterology. 2001;96:1039–46.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=176&id=22&aid=1582