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Protective Role of Crude *Aloe vera* Gel Against Gastric Ulcers in Alloxan - Induced Diabetic Rats

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ABSTRACT

Diabetes Mellitus (DM) has been observed to cause altered acid secretion and increase risk of gastric ulceration. This study was therefore designed to investigate the effect of DM on ulcerogenic parameters and to ascertain the impact of treatment with crude *Aloe vera* gel on same. Twenty male albino Wistar rats weighing 180 - 200 g were randomly assigned one of 4 groups such that each group contained 5 rats, thus control, diabetic untreated group (DM), diabetic group; treated with 0.4 ml/100g crude *Aloe vera* gel (DMT) and control group; treated with 0.4 ml/100g crude *Aloe vera* gel (CT). All the animals had access to food and water *ad libitum*. At the end of 21 days of extract administration, gastric ulcer was induced using standard methods, followed by determination of gastric acid output, gastric mucus and gastric ulcer scores. Basal acid output was significantly ($P<0.05$) increased in DM group, compared to control, while a significant ($P<0.001$) reduction was observed in DMT and CT group compared to control. In response to histamine administration, gastric acid output was highest in DM group and significantly ($P<0.05$) lower in CT group, compared to control. Mucus secretion was significantly ($P<0.001$) reduced in DM group, compared to control. Mucus secretion was significantly ($P<0.01$, $P<0.001$, $P<0.001$) increased in CT group compared to control, DM and DMT group. Ulcer scores was significantly ($P<0.01$) higher in DM group, compared to DMT group. CT group had a significantly ($P<0.01$) lower ulcer score compared to control. We therefore conclude that crude *Aloe vera* gel ameliorates ulcerogenic activities induced by diabetes mellitus by reducing gastric acid secretion and increasing mucus secretion.

Keywords: *Aloe vera* gel, diabetes mellitus, gastric acid, gastric mucus, gastric ulcers

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INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disorder that is characterized by hyperglycemia [1]. It occurs when there is inefficient insulin production as in type 1 diabetes mellitus (T1DM) or when the body cells cannot utilize the insulin produced by the pancreatic beta cells, as in type 2 diabetes mellitus (T2DM). Diabetes is characterized by increased generation of reactive oxygen species and decreased antioxidant levels in the body [2]. The disease has reached an epidemic proportion, and is believed to be responsible for initiating and aggravating many gastrointestinal disorders, gastric ulcers inclusive [3].

Gastric ulcer, also called stomach ulcer, simply refers to perforations in the normal gastric mucosa. Gastric ulceration occurs when there is an imbalance between gastro - protective and gastro - aggressive factors [4]. The rate of gastric acid secretion is a major determinant of the possibility of gastric ulceration. Other factors include rate of mucus secretion (a protective factor), rate of proliferation of gastric cells among others. The incidence of gastric ulcer varies with age, sex and environment [5,6].

The use of herbs or other materials of plant origin is being promoted for a large variety of conditions. Often, general practitioners seem to know less than their patients about the alleged health benefits of plant materials. Aloe is a succulent perennial plant with over 300 species. It belongs to family Liliaceae, native to North Africa and cultivated in warm climatic areas [7]. The most widely used species of Aloe is *Aloe vera* barbadensis. Researchers have named it the most therapeutically effective species of Aloe [8]. Aloe can be utilized therapeutically, in three forms – gel, latex and a combination of the gel and the latex. Aloe latex contains the anthraquinone glycosides - aloin A and B, which are potent laxatives [9,10]. *Aloe vera* has been found to be effective in managing various health conditions in the following body systems - cardiovascular, endocrine, respiratory, gastrointestinal, blood and immune system [1,11-13].

Studies on the effect of DM on gastric ulcers have been inconclusive. Some studies have reported an increase in acid output with a tendency of developing peptic ulcer in DM [14,15], while some other studies have reported no difference in gastric acid secretion in diabetic compared to non-diabetic conditions [16,17].

Following reports on the beneficial effects of crude *Aloe vera* gel in the treatment of T1DM [1], it became necessary to ascertain the impact of treating T1DM with crude *Aloe vera* gel on gastric acid secretion and adherent gastric mucus, which are important determinants of the degree of gastric ulceration in DM.

MATERIALS AND METHODS

Plant Material and Preparation of Crude *Aloe vera* Gel

Mature *Aloe vera* plant with leaves 40 - 60 cm long was obtained from University of Uyo, botanical garden and was authenticated by the Chief Herbarium Officer of Botany Department of University of Calabar, Calabar, Nigeria. The leaves were rinsed with clean water and dried with a clean piece of cloth. A knife was then used to slice the leaf

longitudinally to expose the gel. The gel was gently scraped into an electric blender to shatter the block. Care was taken not to scrape too deep into the leaf to avoid the *Aloe vera* latex which is completely different from the gel. The dose of the crude extract used for this study was 0.4 ml/100g body weight [1].

Animal Preparation and Experimental Grouping

Twenty (20) male albino Wistar rats weighing 180 - 200 g were obtained from the animal house of Pharmacology Department, University of Calabar. After 14 days of acclimatization, the animals were randomly assigned one of four groups, such that each group contained 5 animals. Group 1 served as control; group 2 served as diabetic untreated group (DM), group 3 served as diabetic treated group (DMT) and group 4 served as control treated group (CT). The animals were kept in well ventilated metabolic cages, exposed to 12/12 light/dark circle and allowed access to food and water *ad libitum.*

Induction of Type 1 Diabetes Mellitus

Type 1 diabetes mellitus (T1DM) was successfully induced by a single intraperitoneal administration of alloxan at a dose of 120 mg/kg. The animals were fasted for 12 hours prior to alloxan administration. Diabetes was confirmed 48 hours after alloxan administration by symptoms such as polyuria, polydipsia and polyphagia. Actual glucose concentrations were obtained using a glucose meter (IMFOMED IMPEX INDIA) and test strips. Blood for this purpose was obtained by pricking the distal end of the tail. Animals with fasting blood glucose concentration $\geq$ 180 mg/dl were considered diabetic and used for this study.

Extract Administration

After 14 days of habituation, the crude extract was administered to the DMT and CT group at a daily oral dose of 0.4 mg/100g for 21 days. Administration was facilitated by the use of a syringe and orogastric tube.

Measurement of Gastric Acid

Continuous perfusion method of Gosh and Schild [18] as modified by Osim *et al.* [19] was adopted in measurement of gastric acid secretion. All animals were fasted for 24 hours prior to the start of the experiment. The animals were then anaesthetized by intra-peritoneal administration of 6 ml/kg of 25 % (v/v) solution of urethane (Sigman, UK). The trachea was exposed and cannulated to allow for adequate penetration of oxygen into the lungs. Another cannula was passed into the stomach, through the mouth and then the esophagus. Both cannula were tied firmly in place with a ligature. The abdomen was then cut open 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 the stomach contents. 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 perfused continuously with normal saline at the rate of 1 ml/min.

The pH of the saline was maintained at 7.0 and the body temperature of the rat was maintained at 37 ^{o}C using a heating lamp and a rectal thermometer used to monitor its body temperature. The flow was adjusted to give an effluent volume of about 1 ml per minute. The effluent was collected at 10 minutes interval and care was taken not to ligate the blood vessels as this may lead to stained perfusate. Each perfusate obtained after 10 minutes was titrated using two drops of phenolphthalein as indicator against 0.01 N NaOH (May and Baker, UK) to determine its total acidity. The experiments were repeated using histamine as acid secretagogue, administered subcutaneously. The dose of histamine used was 100 mg/kg body weight. Gastric acid output in the effluent sample was measured by titrimeric analysis. The experiments were also repeated using cimetidine as a blocker at a dose of 100 mg/kg body weight. Administration was via intramuscular route.

Measurement of Gastric Mucus

The adherent gastric mucus was measured by the method of Tan *et al* [20]. The animals were fasted for 18 hours prior to the experiment, after which they were sacrificed and their stomachs removed. The stomachs were then opened along the greater curvature and pinned on a flat board by using pins. By using a spatula, the gastric mucus was scraped off the surface of the mucosa and introduced into a pre-weighed sterilized sample bottle containing 3 ml of distilled water. The sample bottle containing distilled water and the collected mucus was now weighed on a sensitive electronic balance. Mucus output was calculated as the difference in weights of sample bottle containing water and sample bottle containing water and mucus.

Gastric Ulceration

Prior to gastric ulceration, the animals were starved for 24 hours. Under anaesthesia (6 ml/kg of 25 per cent v/v solution of urethane), a pyloric incision was made and a cannula inserted and held in place by tying with a thread. The stomach was infused with 1.5 ml of acid alcohol, prepared from equivolume of 0.1N HCl and 70 % methanol [21]. The infusion was made via the pyloric incision. The animal was left to stay for 1 hour. The stomach was then isolated, washed, cut open along the greater curvature and rinsed with normal saline. Pins were used to hold the tissue to the dissecting board. A magnifying lens and a vernier caliper were used to measure the extent of ulceration. Scoring of ulcer spots was done by the method of Adeniyi and Olowokorun [21] and Alphin & Wards [22]. Digital photographs were taken. Specimens representing each group is shown in plate 1, 2, 3 and 4 for control, DM, DMT and CT group respectively.

STATISTICAL ANALYSIS

The results are presented as mean $\pm$ standard error of mean. The data was analyzed using One - way analysis of variance (ANOVA), followed by the least significant difference (LSD) procedure for significant F values. P = .05 was considered significant. Computer software SPSS and excel analyzer were used for the analysis.

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Representative Photographs in the Different Experimental Groups

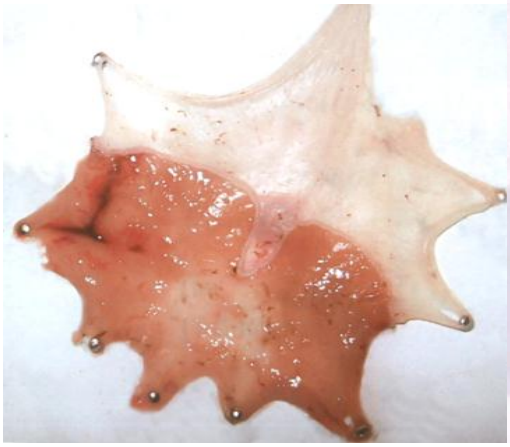

Plate 1

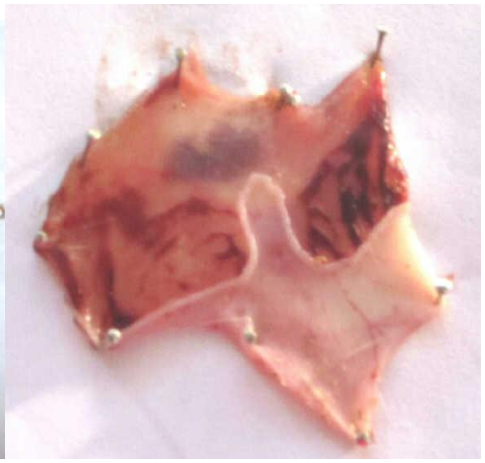

Plate 2

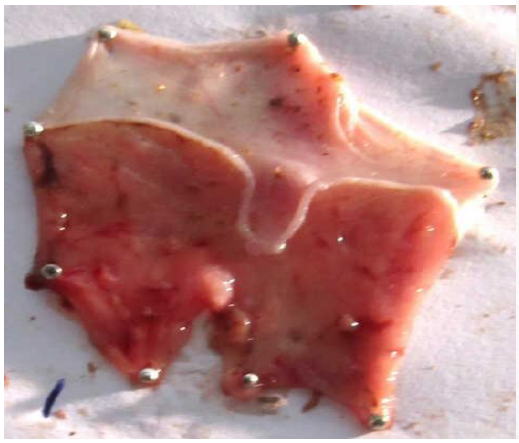

Plate 3

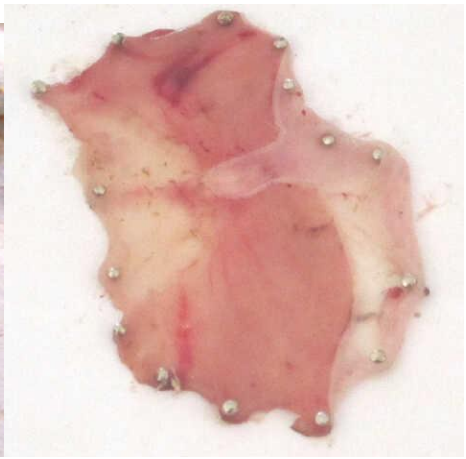

Plate 4

Plate 1: Control group
Plate 2: Diabetic untreated (DM) group
Plate 3: Diabetic treated (DMT) group
Plate 4: Control treated (CT) group

RESULTS

Comparison of Fasting Blood Glucose Concentration in the Different Experimental Groups

There was no significant difference in fasting blood glucose (FBG) concentration in the different experimental groups prior to alloxinization. Forty eight hours after alloxan administration, the FBG concentration for control, DM, DMT and CT group was 64 $\pm$ 1.9, 181 $\pm$ 1.9, 182 $\pm$ 1.7 and 63 $\pm$ 1.4 mg/dl respectively. FBG was significantly ($P<0.001$) higher in DM and DMT group compared to control and CT group (Fig. 1). At the end of 21 days of extract administration, FBG in the control, DM, DMT and CT group was 65 $\pm$ 2.0, 177 $\pm$ 2.1, 65 $\pm$ 2.3 and 62 $\pm$ 1.5 mg/dl respectively. FBG was significantly ($P<0.001$) reduced in DMT group, compared to DM group (Fig. 1).

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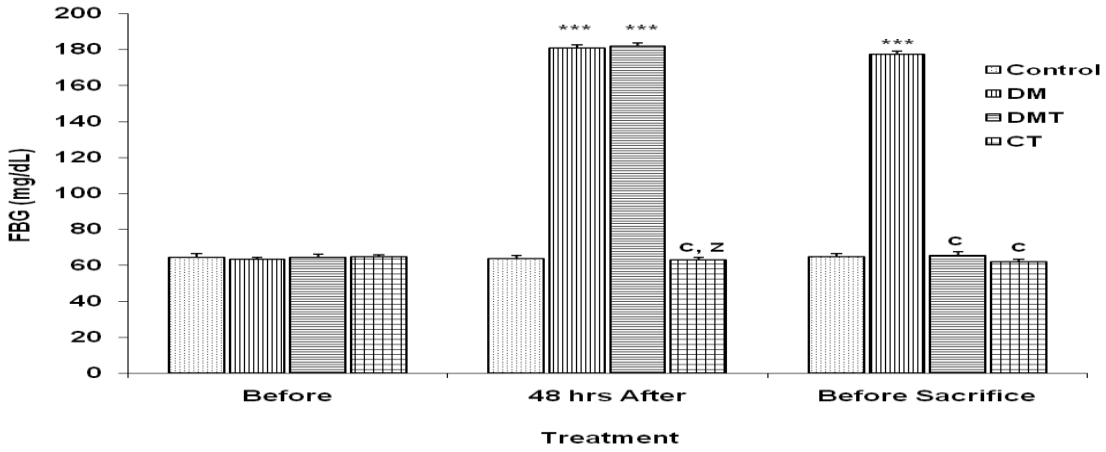

Figure 1. Comparison of fasting blood glucose concentrations in the different experimental groups. values are mean ± SEM, n = 5.
*****p<0.001 vs control; c = p<0.001 vs DM; z = p<0.001 vs DMT.**

Comparison of Basal, Histamine - Induced and Histamine + Cimetidine Induced Gastric Acid Output

The mean basal gastric acid output in the control group, DM, DMT and CT group was 13.1 ± 0.96, 16.2 ± 1.4, 9.4 ± 0.9 and 9.2 ± 0.2 µmol/10mins respectively. Basal acid output was significantly (P<0.05) increased in the DM group, compared to control. Basal acid output was significantly (P<0.001, P<0.05) reduced in DMT and CT groups compared to DM group and control respectively (Fig. 2). After administration of histamine, the mean gastric acid output was 34.2 ± 5.0, 41.4 ± 7.8, 29.72 ± 3.7 and 22.4 ± 2.5 µmol/10mins for control, DM, DMT and CT group respectively. Mean gastric output in CT group was significantly (P<0.05) lower compared to control (Fig. 2). When cimetidine (histamine blocker) was introduced, the mean gastric acid output was 9.3 ± 1.7, 13.2 ± 2.5, 9.8 ± 1.7 and 8.5 ± 1.0 µmol/10mins for control, DM, DMT and CT group respectively. Mean gastric acid output was highest in DM group (Fig. 2).

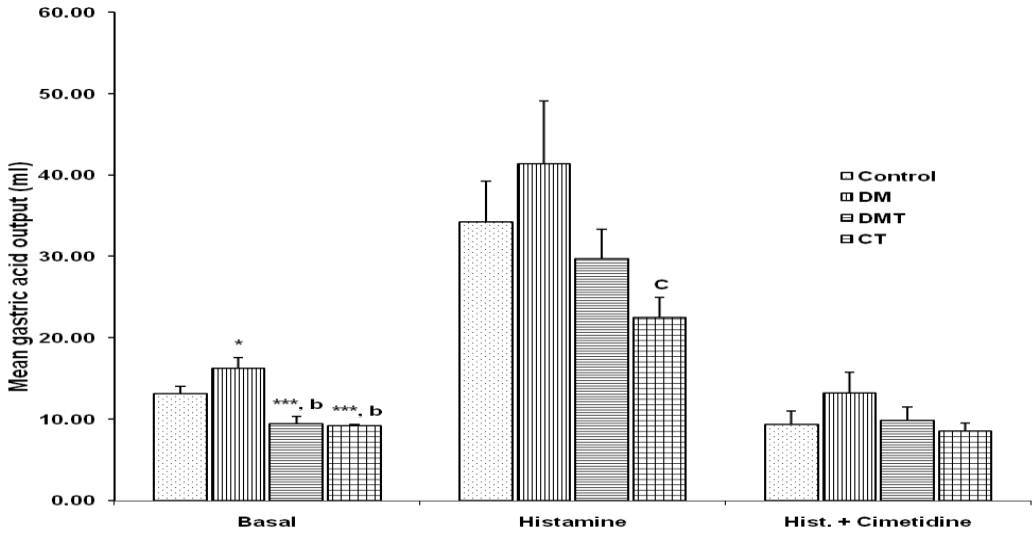

Figure 2a. Comparison of mean gastric acid output in the different experimental groups. Values are mean ± SEM, n = 5.
***p<0.05 vs control; ***p<0.001 vs DM; b = p<0.05 vs control; c = p<0.05 vs control.**

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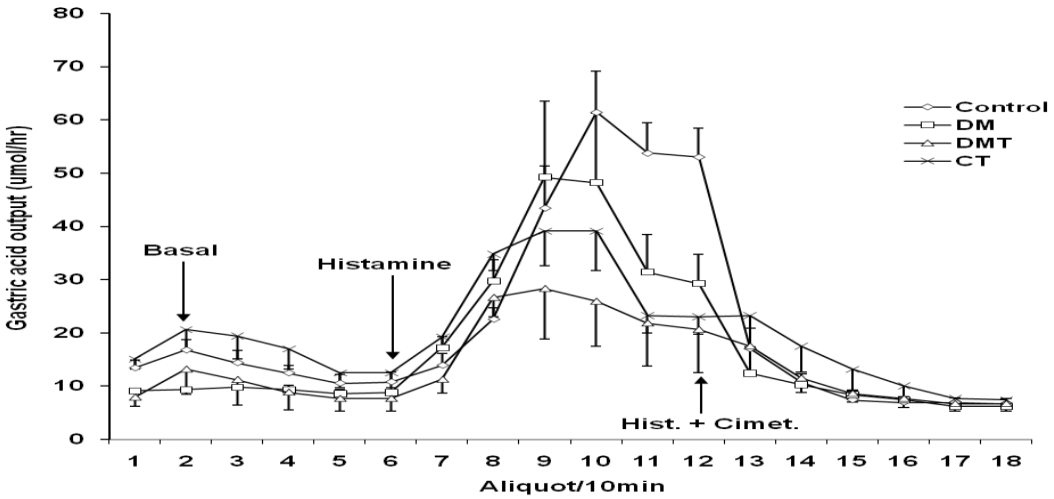

Figure 2b. Comparison of mean gastric acid output in the different experimental groups. Values are mean ± SEM, n = 5.

Comparison of Gastric Mucus Secretion (Weight) in the Different Experimental Groups

The mean weight of gastric mucus was 0.13 ± 0.01, 0.06 ± 0.01, 0.11 ± 0.01 and 0.23 ± 0.04 g for control, DM, DMT and CT group respectively. The mean weight of gastric mucus was significantly (P<0.001) reduced in DM group compared to control. Gastric mucus weight was significantly (P<0.01, P<0.001, P<0.001) increased in CT group compared to control, DM and DMT groups respectively (Fig. 3).

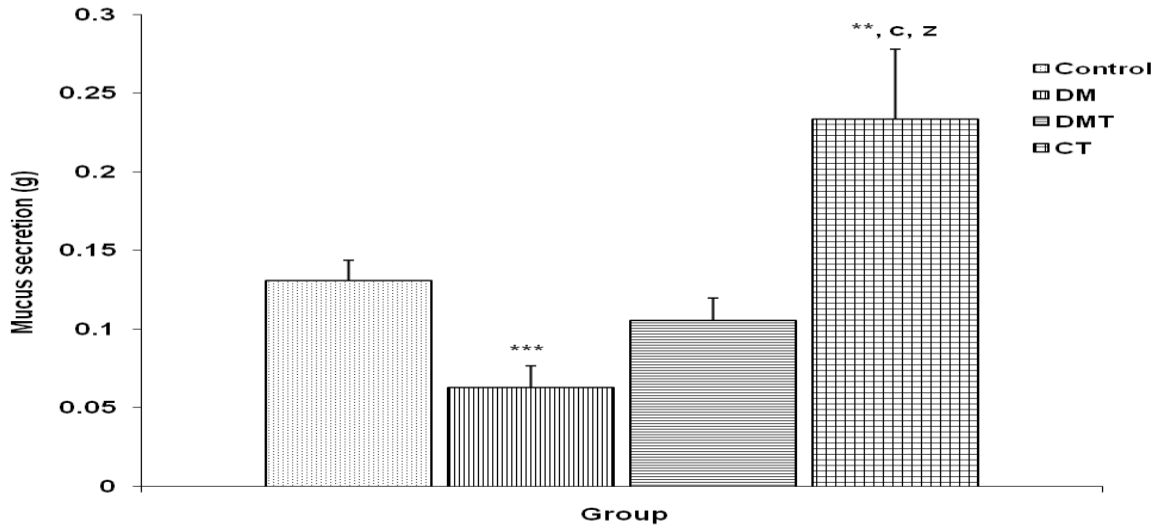

Figure 3. Comparison of gastric mucus secretion in control and test groups. Values are mean ± SEM, n = 5. **p<0.01, ***p<0.001 vs control; c = p<0.001 vs DM; z = p<0.001 vs DMT.

Comparison of Ethanol - Induced Ulcer in the Different Experimental Groups

The mean ulcer score for control, DM, DMT and CT group was 10.9 ± 2.2, 14.4 ± 1.39, 5.60 ± 1.0 and 6.3 ± 1.1 respectively. Mean ulcer score was significantly (P<0.01, P<0.001) reduced in DMT and CT groups respectively, compared to control, and significantly

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(P<0.001) reduced in DMT and CT groups compared to DM group. It was also significantly (P<0.05) increased in DM group compared to control (Fig. 4).

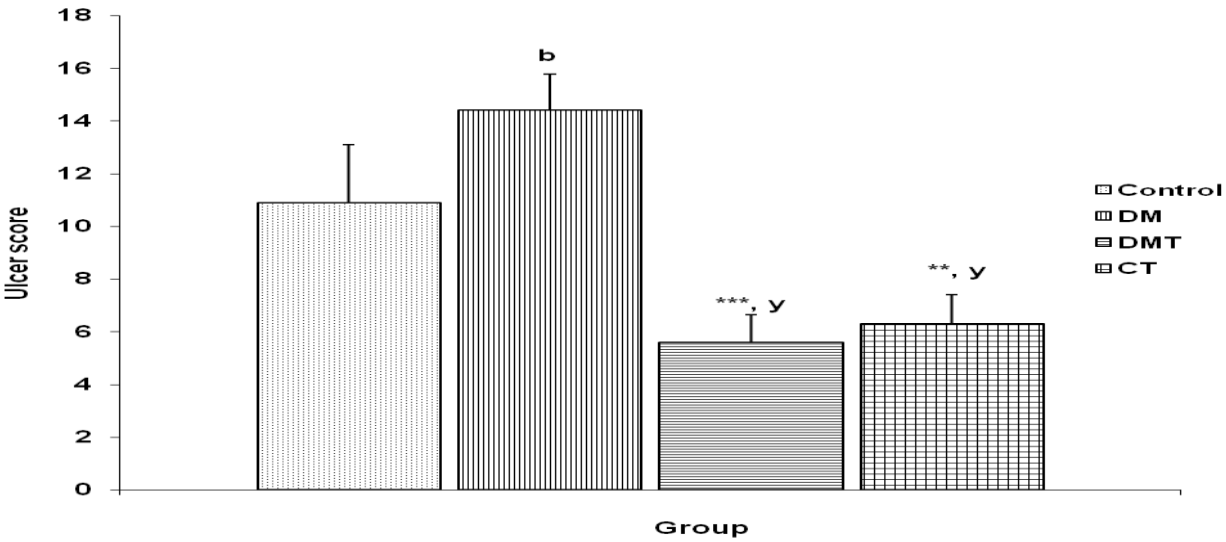

Figure 4.Comparison of ulcer score in control and test groups. Values are mean ± SEM, n = 5. **p<0.01, ***p<0.001 vs control; b = p<0.05 vs control; y = p<0.001 vs DM.

DISCUSSION

This study demonstrated the beneficial effect of crude *Aloe vera* gel on gastric ulcers in T1DM. Fasting blood glucose was significantly reduced in DMT group, compared to control. This effect was previously reported [1].

Basal acid output was significantly increased in DM group, compared to control group. This is contrary to some previous reports that basal acid secretion was reduced in DM [23,24,25]. Basal acid output was significantly reduced in DMT and CT groups compared to DM and control group respectively. Administration of histamine significantly increased acid secretion in all the groups studied. However, CT group had a significantly lower acid secretion compared to control in response to histamine administration. Although gastric acid output was reduced in DM, DMT and CT group when cimetidine (histamine blocker) was introduced, it was not significantly different from control, (Fig. 2a and 2b). The inhibitory effect of *Aloe vera* gel on gastric acid secretion may be due to the presence of lectins in the plant gel [26]. Lectins are proteins/glycoproteins capable of recognizing and binding to carbohydrate moieties [27]. Healey *et al* [28] showed that lectins inhibit aminopyrine uptake by parietal cells, thereby reducing gastric acid secretion. Thus, the ability of crude *Aloe vera* gel to inhibit gastric acid output maybe as a result of direct action of lecitin on the acid producing cells (parietal cells).

Mucus secretion was significantly reduced in the DM group compared to control, (Fig. 3). A condition attributable to the loss of mucus secreting cells lining the stomach wall due to ulceration. Also, the reduced levels of anti - oxidant associated with DM may be responsible for this development. Mucus secretion was increased in DMT group compared to DM group. Mucus secretion was highest in the CT group; there was a significantly higher

mucus secretion in CT group compared to control. This may be attributed to the anti - oxidant and cell regenerative effects of crude *Aloe vera* gel [29,30].

Representative photographs of the groups studied in this report showed that ulceration was more in the DM group (Plate 2), compared to control (Plate 1). Diabetes induces cellular and functional changes in the glandular stomach especially in the parietal cells. Changes such as the decrease in the number of mitochondria accompanied by reduction in H^+ - K^+ ATPase and canaliculi in parietal cells was earlier reported [25]. Destruction of parietal cells (which secrete HCl) maybe due to the reduction in gastric mucus secretion observed in the DM group (Fig. 4). Gastric ulceration was significantly reduced in DMT (Plate 3) and CT (Plate 4) group compared to DM and control group respectively, following an increase in mucus secretion. Although helicobacter pylori infection is currently regarded as the main causative factor for gastritis and is responsible for the vast majority of peptic ulcer, it is evident from our observations that diabetes is a risk factor in the development of gastric ulcer [31].

In a study conducted by Rodriguez and Cruz [32] on guinea pig, *Aloe vera* gel extract permitted faster healing of burn, and re - established the vascularity of destroyed tissues. These effects may be due to an increasing collagen synthesis, an increasing rate of epithelization by the effect of acemanan (mannose-6 phosphate) to stimulate fibroblasts, an antimicrobial effect and/or an anti-inflammatory effect [32,33,34].

CONCLUSION

On the basis of the results obtained from this study, we therefore conclude that crude *Aloe vera* gel reduces gastric ulceration by reducing gastric acid secretion and increasing gastric mucus secretion in normal and diabetic conditions. This makes it beneficial in the treatment of gastric ulcers secondary to T1DM.

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