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Effect of *Aloe vera* gel on Small Intestinal Motility and Transit in Alloxan - Induced Diabetic Rats.

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Research Article

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ABSTRACT

The use of *Aloe vera* gel in the management of Diabetes Mellitus (DM) is widely known in Nigeria. Since DM is associated with altered small intestinal motility and transit, this study therefore seeks to ascertain the impact of treatment of type 1 diabetes mellitus (T1DM) with *Aloe vera* gel on small intestinal motility and transit. Thirty two male albino wistar rats weighing 180 - 200 g were randomly assigned 1 of 4 groups, with group 1 serving as control, group 2 - test 1 (diabetic untreated), group 3 - test 2 (diabetic treated) and group 4 - test 3 (treated control). The dose of *Aloe vera* gel used was 0.4 ml/100g daily, per oral route. After 21 days of administration, animals were sacrificed and intestinal motility and transit experiments were carried out on the isolated rat ileum using appropriate methods. Acetylcholine (ACh) and atropine were used to challenge the isolated rat ileum. Results obtained showed that test 1 had altered pattern of small intestinal motility compared to control when ACh was used to challenge the rat ileum. Test 2 showed a contraction pattern similar to that of control, while test 3 recorded a rather reduced contraction compared to control. In response to atropine, percentage relaxation in test 3 was significantly ($p<0.01$, $p<0.001$, $p<0.001$) higher compared to control, test 1 and 2 respectively. Percentage transit in test 1 and 3 was significantly ($p<0.001$) reduced compared to control. It was also significantly ($p<0.05$) reduced in test 2 compared to test 1. We therefore conclude that *Aloe vera* gel mitigates the altered small intestinal motility caused by T1DM, but potentiates the reduction in small intestinal transit caused by T1DM.

INTRODUCTION

The prevalence of altered gastrointestinal functions in diabetes has become a matter of great concern the world over. Alterations in the pattern of gastrointestinal (GI) motility in diabetes mellitus (DM) so far reported has not been consistent, ranging from marked dysmotility [1,2,3], to increased motility [4,5,6,7], to normal motility [8]. Some studies have linked the altered GI motility observed in DM to changes in exogenous glucose load, either from the meal or from hepatic glucose production [9,10,11].

Some studies have shown that small intestinal transit time depends partly on the amount of the fluidly component of a meal. In DM however, the factor influencing the GI motility pattern seems to revolve round the increased glucose load in the small intestine resulting from polyphagia [12,13]. These studies explained the fact that blood glucose levels within the range 12 - 15 mmol/L reduced the number and propagation of pressure waves in the proximal small intestine [14], resulting in increased small intestinal transit time [15]. Studies using different experimental animal models of diabetes have shown altered activity of many neurotransmitters like serotonin, calcitonin-related peptide, substance P, peptide Y and NO, known to be instrumental in maintaining the integrity of intestinal motility [16,17]. These observations, together with alterations in the release of various gastrointestinal hormones are known to be regulated by the amount of glucose entering the duodenum.

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The use of *Aloe vera* gel for treatment of various ailments has been widely documented. *Aloe vera* is a succulent perennial plant that belongs to family Liliaceae, with over 350 species [18]. *Aloe vera* gel which is visible on slicing the leaf has been reported to be beneficial in T1DM, atherosclerosis, asthma and in wound healing [19-23]. *Aloe vera* latex which is obtained from the inner part of the skin of the leaves has been proven to contain anthraquinones and possess laxative effect [24].

Considering the importance of small intestinal motility and transit to the magnitude of absorption of glucose and other nutrients, it became necessary to ascertain the impact of treatment of T1DM with *Aloe vera* gel on small intestinal motility and transit, which are important determinants of the magnitude of absorption of products of digestion.

MATERIALS AND METHOD

Plant Material and Extract Preparation

Fresh *Aloe vera* leaves of 40 - 50 cm length were obtained from the University of Uyo botanical garden and identified by the Chief Herbarium Officer of the department of Botany, University of Uyo, Akwa Ibom State, Nigeria. The leaves were rinsed in clean water to remove debris and sand, after which they were dried using a clean cloth. Using a knife, the leaves were sliced longitudinally to expose the gel. The gel was gently scraped into an electric blender to shatter the block. Adequate care was ensured to avoid scraping too deep into the *Aloe vera* skin to avoid the juice from the leaf which is entirely different from the gel. This preparation was done daily and given to the animals without storage to prevent contamination secondary to prolonged storage. The median lethal dose of the plant material was determined by method of Lorke [25].

Experimental Animals and Protocol

Thirty two male albino Wistar rats weighing 180 - 200 g were used for this study. The animals were placed in well ventilated cages and were exposed to normal temperature and 12/12 hours light/dark cycle. After fourteen days of habituation, the animals were randomly divided into 4 groups of 8 animals each. The groups were labeled as follows; group 1 - control, group 2 - test 1 (diabetic untreated group), group 3 - test 2 (diabetic treated group) and group 4 - test 3 (control treated group). The dose of *Aloe vera* gel used for this study was 0.4 ml/100g body weight. All animals had access to food and drinking water *ad libitum*.

Induction of Type 1 Diabetes Mellitus

Animals in test groups 1 and 2 were deprived of food, but allowed access to drinking water 18 hours prior to diabetes induction. Type 1 diabetes mellitus (T1DM) was successfully induced by intra - peritoneal administration of alloxan at a dose of 120 mg/kg. Alloxan was administered immediately after preparation to prevent oxidation due to long standing. Symptoms (polyuria, polyphagia and polydipsia) of T1DM were observed 48 hours after alloxan administration. Diabetes was confirmed using a Glucometer (ACCU-CHECK Advantage II, Roche Diagnostics GmbH, Germany) and ACCU-CHECK Advantage II test strips. Blood used for blood glucose measurement was obtained from the distal part of the animal's tail. Animals with fasting blood glucose level $\geq$ 180 mg/dl were considered diabetic and selected for this study.

Extract Administration

Extract administration commenced 4 days after successful diabetes induction. The extract was administered to test groups 2 and 3 at a dose of 0.4 ml/100g, once daily for 21 days. Administration was facilitated by the use of a syringe and orogastric tube. All experiments regarding the animals and their care were in line with approved standards of the local ethics committee.

Small Intestinal Motility Determination

Animals were starved for 24 hours prior to small intestinal motility experiment. The animals were stunned, then sacrificed. An incision was quickly made through the linea alba to expose the intestine. The proximal ileum was identified and isolated, then placed in a container of tyrode solution and aerated. The ileum was then cut into small segments of 3 cm in length, and mounted at one end to a fixed support in an organ bath. The other end of the ileum was fixed to a horizontal balance writing lever tangential to a kymograph drum. The tissue was allowed to equilibrate for 60 minutes. Within the 60 minutes, the bathing solution was replaced with tyrode solution at 15 minutes interval to avoid accumulation of metabolites. The tissue was then challenged with graded doses of acetylcholine (10^{-3} to 10^{-9} mg) and later with atropine (0.1 mg), at an interval of 1 minute/administration.

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Determination of Small Intestinal Transit

Small intestinal transit was determined using the method of Uwagboe and Orimilique [26]. The rats in the different experimental groups were starved for 24 hours prior to the experiment, but had unrestricted access to drinking water. 10 g of activated charcoal was thoroughly mixed with 1 g gum Arabic in 100 ml of distil water to serve as the marker substance. Each animal was gavaged with 2 ml of the marker substance, orally using a metallic (8 cm long) intubating syringe. The animals were timed for 60 minutes each, after which they were sacrificed by cervical decapitation. The abdomen was immediately cut open through the linea alba to minimize bleeding. The duodenum was then identified as the continuation of the pyloric sphincter while the ileocecal sphincter was also prominent at the cecal end. The duodenum was cut away from the pyloric sphincter and the ileum was also cut at the ileocecal sphincter. The small intestine was immediately straightened and the location of the marker was identified along the small intestine. A thread was used to tie the intestine at the point where the marker stopped. Using a measuring tape, the total length of the small intestine was measured and recorded. The length travelled by the marker was also measured and recorded. The small intestinal transit was calculated as:

$$\frac{\text{Length travelled by marker substance}}{\text{Total length of small intestine}} \times 100$$

The values were recorded and statistically analyzed.

Statistical Analysis

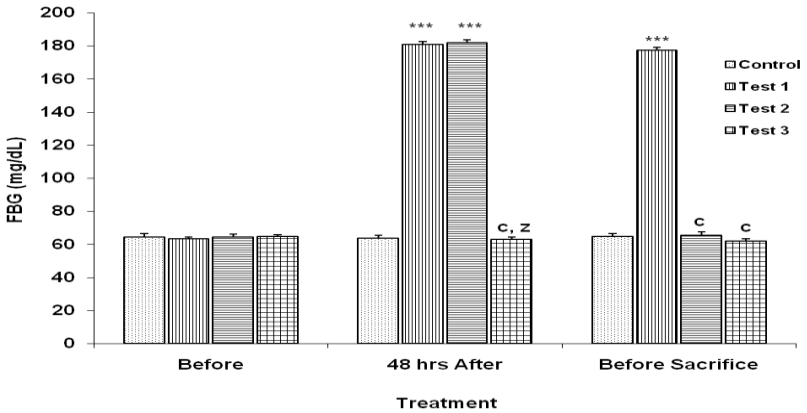
The results are presented as mean ± standard error of mean (SEM). The results were analyzed using one way analysis of variance (ANOVA), followed by the least significant difference (LSD) procedure for significant F values, P = .05 was considered significant. Computer software SPSS and Excel Analyzer were used for the analysis in this study.

RESULTS

Fasting Blood Glucose Measurement in the Different Experimental Groups

Measurement of fasting blood glucose level in the different experimental groups prior to diabetes induction revealed that there was no significant difference in the fasting blood glucose level of the animals before alloxan administration. Forty eight hours after alloxan administration, the blood glucose level in the control, test 1, test 2 and test 3 was 64 ± 1.9, 181 ± 1.9, 182 ± 1.7 and 63 ± 1.4 mg/dl respectively, (Fig. 1). The fasting blood glucose level was significantly increased ($p<0.001$) in test 1 and 2 compared to control and test 3. After 21 days of extract administration, the fasting blood glucose level in the control, test 1, test 2 and 3 was 65 ± 2.0, 177 ± 2.1, 65 ± 2.3 and 62 ± 1.5 mg/dl respectively. Fasting blood glucose level was significantly reduced ($p<0.001$) in test 2 compared to DM group (Fig. 1).

Figure 1: Comparison of fasting blood glucose concentrations in control and tests groups before and after alloxan administration.



Values are mean ± SEM, n = 8.
***p<0.001 vs control; c = p<0.001 vs test 1; z = p<0.001 vs test 2

Effect of Graded Doses of Acetylcholine (ACh) on Small Intestinal Motility in the Different Experimental Groups

The response of isolated rat ileum to graded doses of acetylcholine with concentration of 10^{-9} to 10^{-3} g/ml for control, test 1, 2 and 3 is shown in figure 2. At low doses, there was a gradual relaxation of the ileum in diabetic untreated group (test 1), followed by a gradual increase in contractile response of the ileum at a concentration of

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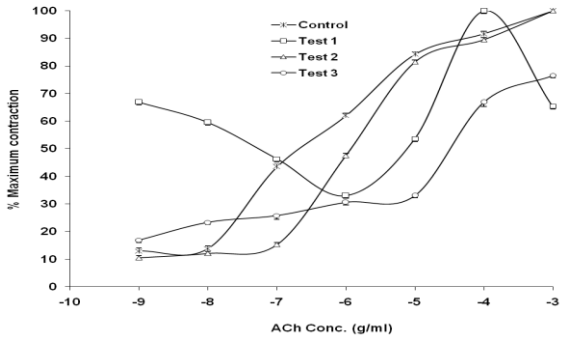
10^{-6}. This was followed by a rapid decrease in contraction at a concentration of 10^{-4}. At low concentrations, there was an increase in isolated rat ileum contraction in the control group, test 2 and 3. The increase in contraction was more in control and test 2 compared to test 3. The isolated rat ileum in test 3 showed a pronounced increase in contraction at a concentration of 10^{-5} (Fig. 2).

Figure 2: Contraction of the rat ileum following administration of graded concentrations of ACh in the different experimental groups.

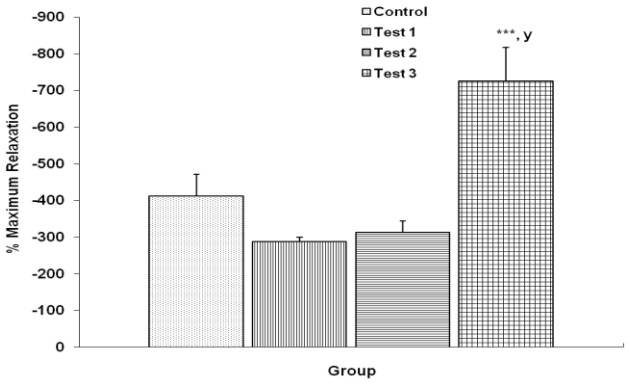


Values are mean ± SEM, n = 8.

Effect of Atropine on Small Intestinal Motility in the Different Experimental Groups

Administration of atropine resulted in an increase in percentage relaxation of the isolated rat ileum in test 3 which was significantly ($p<0.01$) higher compared to control. The percentage relaxation of the isolated rat ileum in test 3 was also significantly ($p<0.001$) higher compared to test 1 and 2 (Fig. 3).

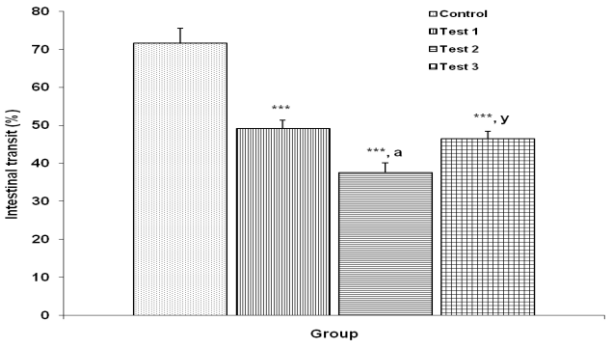
Figure 3: Comparison of effect of atropine on intestinal motility in control and tests groups.



Values are mean ± SEM, n = 8.
y = $p<0.01$ vs control, ***$p<0.001$ vs test 1 and 2

Small Intestinal Transit in the Different Experimental Groups

Figure 4: Comparison of intestinal transit (expressed as percentage of the total length of intestine) in control and tests groups.



Values are mean ± SEM, n = 8.
***$p<0.001$ vs control; a = $p<0.05$ vs test 1; y = $p<0.01$ vs test 2

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The percentage transit for control, test 1, 2 and 3 was 71.74 ± 4.54, 49.25 ± 2.36, 37.54 ± 2.08 and 46.51 ± 2.71 % respectively. Percentage transit was significantly ($p<0.001$) lower in test groups 2 and 3, compared to control. It was also significantly ($p<0.05$) lower in test group 2, compared to test 1. Percentage transit was significantly ($p<0.01$) higher in test 3, compared to test 2 (Fig. 4).

DISCUSSION

Diabetes mellitus has been proven to be associated with persistent hyperglycemia. The measurement of fasting blood glucose concentration 48 hours after alloxinization confirmed T1DM in the groups administered to induce diabetes (test groups 1 and 2). This suggests the possible destruction of pancreatic beta cells in those groups by alloxan - a known cytotoxic drug used to induce experimental diabetes. Fasting blood glucose level was successfully restored to normal control levels by administration of *Aloe vera* gel (Fig. 1). This is consistent with studies conducted by Nna *et al* and Akpan *et al* [19,27] which showed that *Aloe vera* gel reversed T1DM.

Studies on the effect of DM on GI motor functions have been inconclusive because of various discrepancies in reported results. Some have insisted that there are no alterations in the pattern of GI motor activity in DM [6]. Various agents known to affect GI motor activities were used for this study. Administration of ACh to isolated rat ileum resulted in an abnormal pattern of contraction in test group 1, compared to control. The ileum for test 1 showed a gradual decrease in contraction, with the lowest recorded at 10^{-6} g/ml ACh. Test 1 ileum reached its peak of contraction at 10^{-4} g/ml ACh, and later reduced at 10^{-3} g/ml. In contrast, the response of the ileum to ACh administration in test group 2 was consistent with that of control, increasing consistently from 10^{-9} to 10^{-3} g/ml ACh. Although the percentage maximum contraction in test group 3 was higher than that of control at 10^{-9} and 10^{-8}, the control group maintained a steady increase in percentage maximum contraction from 10^{-7} and remained higher throughout the experiment. This observation is contrary to the report of Okon *et al* [28] who noted that DM was associated with a consistent increase in percentage maximum contraction of the isolated rat ileum when challenged with ACh.

Although the percentage maximum relation of isolated rat ileum in test 1 in response to atropine - a cholinergic blocker, was low, it was not significantly different from control. Percentage maximum relaxation was highest in test 3.

Small intestinal transit was significantly ($p<0.001$) reduced in test 1 (diabetic untreated group) compared to control. This suggests a possible role of glucose in reducing intestinal transit in DM. Administration of *Aloe vera* gel to test 2 (diabetic treated group) potentiated the effect of DM on intestinal transit (Fig. 4). This development was confirmed in test 3 (control treated group) which showed a reduced intestinal transit, significantly ($p<0.001$) lower compared to control. Nna *et al* [29] had linked this effect of *Aloe vera* gel on small intestinal transit of normal animals to its high monosaccharide and polysaccharide content which accounts for about 20 percent of its solid composition. The possible mechanism by which this effect is mediated points to a group of gastrointestinal hormones called incretin which are triggered by the presence of glucose rich chyme in the duodenum [30]. Their release results in slowing gastric emptying and reducing small intestinal transit.

CONCLUSION

From the results obtained in this study, we therefore conclude that *Aloe vera* gel mitigates the altered small intestinal motility caused by T1DM, but potentiates the reduction in small intestinal transit caused by T1DM, a development that may favour absorption of digested food materials. However, further studies are required to ascertain the impact of this development on absorption of products of digestion.

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