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Effect of Crude *Aloe vera* Gel on Serum Enzymes, Proteins and Liver Histology in Alloxan - Induced Diabetic Rats

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Abstract: ***Considering the wide use of Aloe vera gel in the management of Type 1 Diabetes Mellitus (T1DM), this study was designed to ascertain the effects of T1DM on serum enzymes and proteins, as well as the impact of treatment with Aloe vera gel on same. Forty male Wistar rats weighing 150 - 180 g were randomly assigned into 4 groups (n = 10), namely, control group; test groups 1 (DM untreated); 2 (DM treated) and 3 (control treated). Diabetes was induced by intraperitoneal administration of 100 mg/kg alloxan. Freshly prepared Aloe vera gel was administered to test groups 2 and 3 at a dose of 0.4 ml/100g per oral route for 21 days. The animals were sacrificed, and serum enzymes and proteins were measured using standard methods. Liver histology was also prepared using standard methods. Serum aspartate aminotransferase (AST) concentration was significantly higher (P<0.001) in test 1, 2 and 3, compared with control. It was also significantly (P<0.001) higher in test 3, compared with test 1 and 2. Serum alanine aminotransferase (ALT) and alkaline phosphatase (ALP) concentrations were significantly reduced (P<0.001) in test 1, compared with control, while in test 3, ALT concentration was significantly increased (P<0.001) compared with control. Serum ALT and ALP concentrations were significantly increased (P<0.001) in test 2, compared with test 1. Serum total protein, albumin and globulin concentrations were significantly reduced (P<0.001) in test 1, 2 and 3, compared with control. Serum total protein concentration was significantly increased (P<0.001) in test 2 and 3, compared with test 1. Serum globulin concentration was significantly reduced (P<0.001) in test 2, compared with test 1. Liver histology was normal in control, test 2 and 3, compared with test 1 which showed grossly abnormal structures. The results obtained in this study revealed that Aloe vera gel mimics T1DM in increasing serum concentrations of liver enzymes, notably, AST and ALT, and reducing serum protein concentrations through a mechanism that may not be related to liver damage.***

Keywords: *Aloe vera,* diabetes mellitus, liver, serum enzymes, serum proteins

1. Introduction

In the world today, cases of diabetes mellitus (DM) and its associated clinical complications are on the rise. Diabetes mellitus is a group of metabolic disorder that is characterized with persistent hyperglycemia [1]. Liver disease may cause or contribute to, or occur as a result of diabetes mellitus. Understanding the role of the liver in the regulation of carbohydrate homeostasis is essential in identifying the many physical and biochemical alterations occurring as a result of diabetes. The liver utilizes glucose as fuel and also has the ability to store same as glycogen synthesizing it from non-carbohydrate precursors (gluconeogenesis). Some studies have demonstrated that a total hepatectomy in a dog resulted in death within a few hours. The authors attributed the death to hypoglycemic shock, underscoring the importance of the liver in maintaining normoglycemia [2,3].

The use of *Aloe vera* gel has been promoted for the management of many disorders. *Aloe vera* is a succulent perennial plant belonging to family *Liliaceae,* having over 350 species [4]. *Aloe vera* gel which is visible on slicing the Aloe leaf has been reported to be beneficial in T1DM [4, 5, 6], atherosclerosis [7] and wound healing [8]. *Aloe vera* latex which is obtained from the inner part of the skin of the leaves has been reported to contain anthraquinones and possess laxative effect [9].

Considering the possible detrimental effects of DM on the state of health of the liver, and the promoted use of *Aloe vera* gel DM management, it became necessary to ascertain the effect of treating DM with *Aloe vera* gel on liver enzymes and serum proteins which are important indicators of the state of health of the liver.

2. Materials and Methods

2.1 Plant Material and Preparation of *Aloe vera* gel

Aloe vera plant with leaves between 40 and 60 cm in length were 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 to remove debris and sand, and thereafter, 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. The dose of the crude extract used for this study was 0.4 ml/100g body weight [5].

2.2 Animal Preparation and Protocol

Forty (40) male Wistar rats weighing 150 - 180 g were used for this study. The animals 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 (n = 10), as follows; Group 1 served as control; group 2 (test 1) served as diabetic untreated group, group 3 (test 2) served as diabetic treated

group and group 4 (test 3) served as control treated group. The animals were housed in well ventilated metabolic cages, exposed to 12/12 light/dark cycle and allowed free access to food and water *ad libitum.*

2.3 Extract Administration

Administration of *Aloe vera* gel began after 14 days of acclimatization. *Aloe vera* gel was administered to test group 2 and 3 at a daily oral dose of 0.4 mg/100g per oral route, for 21 days. Administration was facilitated using an orogastric tube.

2.4 Induction of Type 1 Diabetes Mellitus

Type 1 DM was induced by intraperitoneal administration of alloxan at a single dose of 100mg/kg, after 24 hours fast. Diabetes was confirmed 48 hours after alloxan administration using a glucose meter (IMFOMED IMPEX, INDIA) and test strips. Blood used for this purpose was obtained by pricking the distal end of each animal's tail. Rats with fasting blood glucose level $\geq$ 180 mg/dl were considered diabetic.

2.5 Determination of Serum Enzyme Concentrations

Serum concentration of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were determined by the method described by Reitman and Frankle [10], modified by Bhutia *et al* [11].

2.6 Determination of Serum Protein Concentrations

Plasma protein concentration was measured using reagents from Randox, UK. Total serum protein was assayed by adding 20 µl of serum to 1ml of biuret reagent. The mixture was incubated at room temperature for 10 mins and read at 540 nm. Albumin concentration was measured by adding 10µl of sample to 3 ml of bromocresol green reagent. The mixture was incubated at room temperature for 5 mins and read at 630nm. Values for Globulin were obtained from the difference between total protein and albumin.

2.7 Liver Histology

The animals in the different groups were sacrificed under light anesthesia (diethyl ether) 24 hours after the last administration of *Aloe vera* gel. A small piece of hepatic tissue from the anterior portion of the left lateral lobe was removed and used for histological analysis. The sample was fixed by immersion in 10% neutral buffered formalin. The sample was then embedded in paraffin, sliced into 5 µm sections, and stained with hematoxylin-eosin for histological assessment. The degree of liver tissue damage was evaluated semi quantitatively by method of Jamshidzadeh *et al* [12].

2.8 Statistical Analysis

All results are presented as Mean $\pm$ SEM. One way analysis of variance (ANOVA) was used to analyze the data collected, followed by the post hoc multiple comparison (Least significant difference procedure). $P<0.05$ was considered significant. Excel analyzer was used for the analysis.

3. Results

3.1 Serum Enzymes Concentration

Aspartate Aminotransferase (AST) Concentration

The mean values for AST in the control group, test 1, 2 and 3 were 103.1 $\pm$ 0.64, 174.4 $\pm$ 0.85, 165.7 $\pm$ 0.79 and 243.10 $\pm$ 2.21 IU/L respectively. Serum AST concentration was significantly higher ($P<0.001$) in test 1, 2 and 3, compared with control. It was also significantly ($P<0.001$) higher in test 3, compared with test 1 and 2, (Fig. 1).

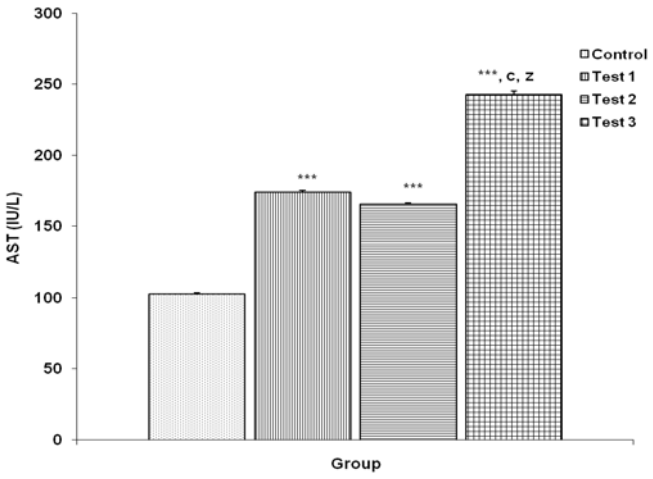

Figure 1: Comparison of aspartate aminotransferase (AST) concentration in control and tests groups. Values are Mean $\pm$ SEM, n = 10. ***p<0.001 vs control; c = p<0.001 vs test 1; z = p<0.001 vs test 2.

Alanine Aminotransferase (ALT) Concentration

The mean values for ALT in the control group, test 1, 2 and 3 were 77.9 $\pm$ 1.69, 72.5 $\pm$ 0.93, 78.0 $\pm$ 0.42 and 93.7 $\pm$ 0.56 IU/L respectively. Serum ALT concentration was significantly reduced ($P<0.001$) in test 1, compared with control, while in test 3, it was significantly increased ($P<0.001$) compared with control. Serum ALT concentration was significantly increased ($P<0.001$) in test 2, compared with control, (Fig. 2).

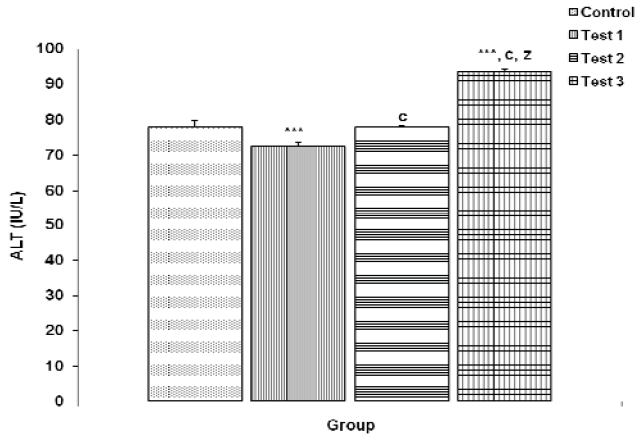

Figure 2: Comparison of alanine aminotransferase (ALT) concentration in control and tests groups. Values are Mean ± SEM, n = 10. ***p<0.001 vs control; c = p<0.001 vs test 1; z = p<0.001 vs test 2.

Alkaline Phosphatase (ALP) Concentration

The mean values for ALP in the control group, test 1, 2 and 3 were 135.6 ± 3.09, 123.5 ± 1.06, 135.1 ± 1.14 and 137.3 ± 1.16 IU/L respectively. Serum ALP concentration was significantly reduced (P<0.001) in test 1, compared with control. Serum ALP concentration was significantly increased (P<0.001) in test 2 and 3, compared with control, (Fig. 3).

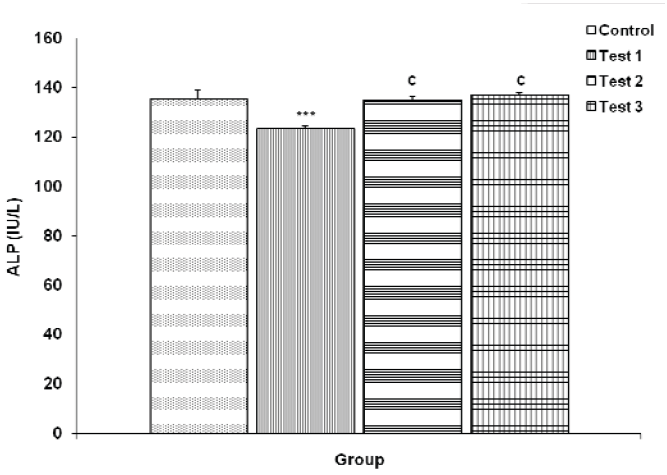

Figure 3: Comparison of alkaline phosphatase (ALP) concentration in control and tests groups. Values are Mean ± SEM, n = 10. ***p<0.001 vs control; c = p<0.001 vs test 1

3.2 Serum Protein Concentration

Serum Total Protein Concentration

The mean serum total protein concentration was 56.6 ± 0.78, 28.6 ± 1.33, 32.3 ± 0.68 and 36.2 ± 0.65 g/L for control, test group 1, 2 and 3 respectively. Serum total protein concentration was significantly reduced (P<0.001) in test 1, 2 and 3, compared with control. It was also significantly increased (P<0.001) in test 2 and 3, compared with test 1. (Fig. 4).

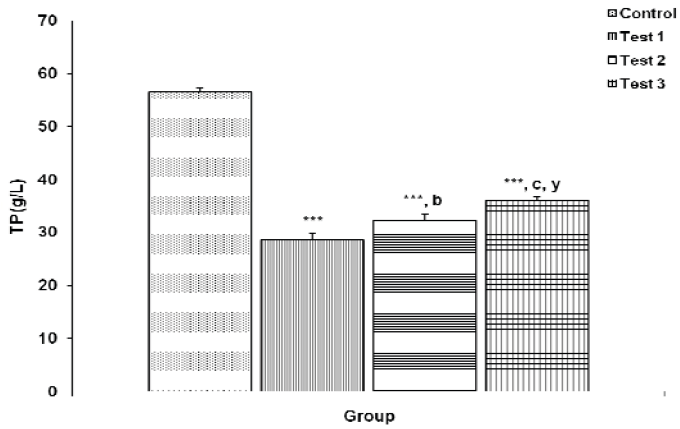

Figure 4: Comparison of total protein (TP) concentration in control and tests groups. Values are Mean ± SEM, n = 10. ***p<0.001 vs control; b = p<0.01, c = p<0.001 vs test 1; y = p<0.001 vs test 2.

Serum Albumin Concentration

The mean serum albumin concentration was 36.4 ± 0.87, 20.3 ± 0.42, 22.8 ± 0.81 and 21.8 ± 0.53 g/L for control, test group 1, 2 and 3 respectively. Serum albumin concentration was significantly reduced (P<0.001) in test 1, 2 and 3, compared with control, (Fig. 5).

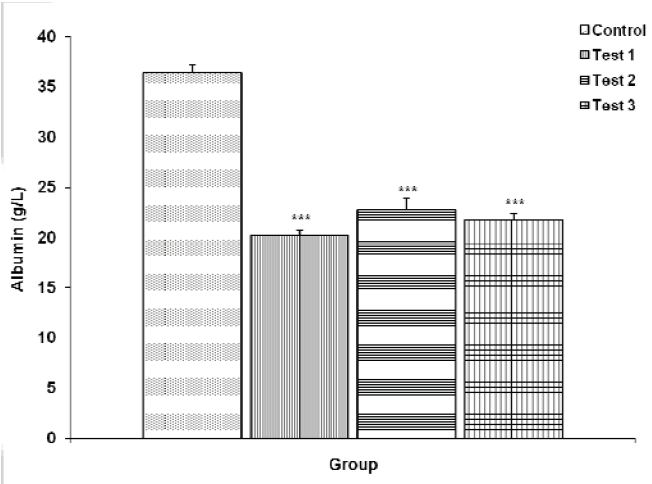

Figure 5: Comparison of serum albumin concentration in control and tests groups. Values are Mean ± SEM, n = 10. ***p<0.001 vs control

Serum Globulin Concentration

The mean serum globulin concentration was 18.8 ± 0.7, 10.0 ± 0.54, 7.8 ± 0.57 and 13.0 ± 0.79 g/L for control, test group 1, 2 and 3 respectively. Serum globulin concentration was significantly reduced (P<0.001) in test 1, 2 and 3, compared with control. It was also significantly reduced (P<0.001) in test 2, compared with test 1. (Fig. 6).

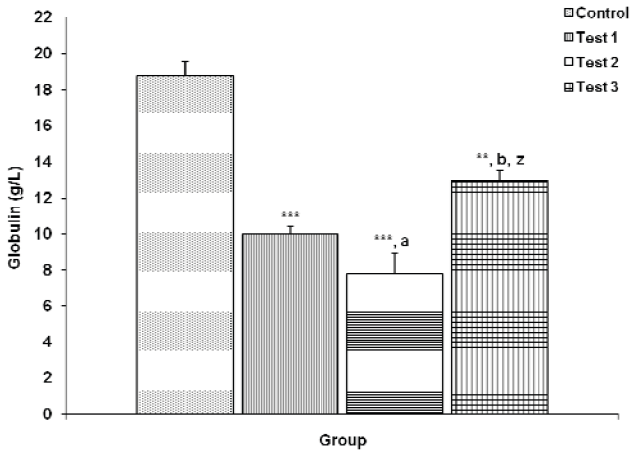

Figure 6: Comparison of serum globulin concentration in control and tests groups. Values are Mean ± SEM, n = 10. ***p<0.001 vs control; a = p<0.05, b = p<0.01 vs test 1; z = p<0.001 vs test 2.

3.3 Liver Histology

Representative plates for all four groups are shown below. H & E staining technique was used with magnification 100x.

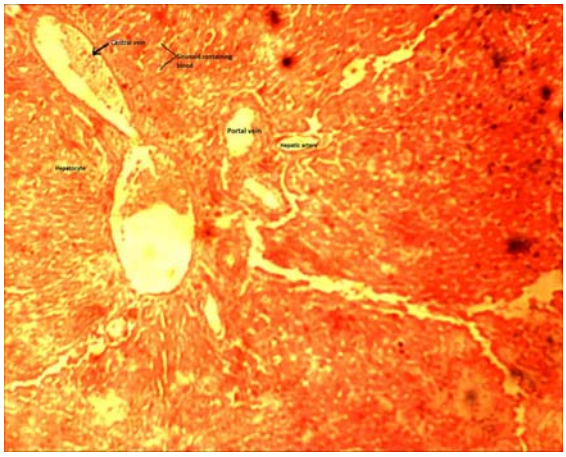

Plate 1: Control: the normal histological structures are retained without defect on the portal triad and sinusoid distribution.

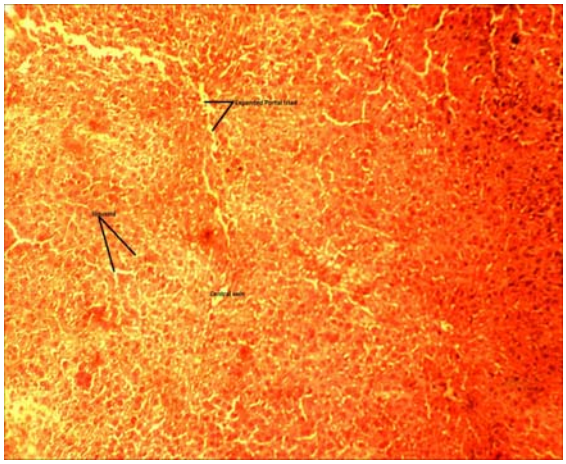

Plate 2: Test 1 (T1DM untreated group): The sinusoid is dilated; the portal triad is equally dilated with congested blood vessels.

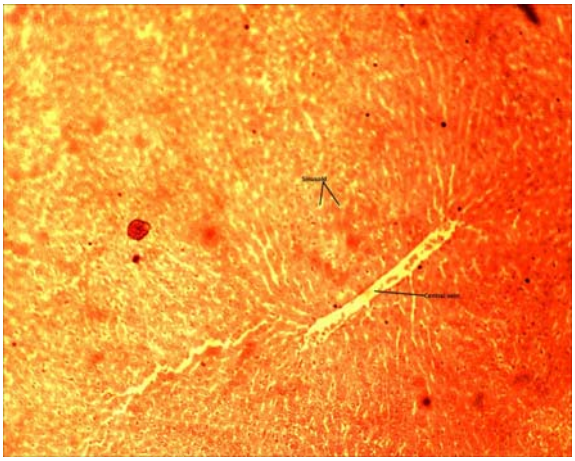

Plate 3: Test 2 (T1DM treated group): The sinusoid is not dilated, normal portal triad distribution with no inflammatory cells.

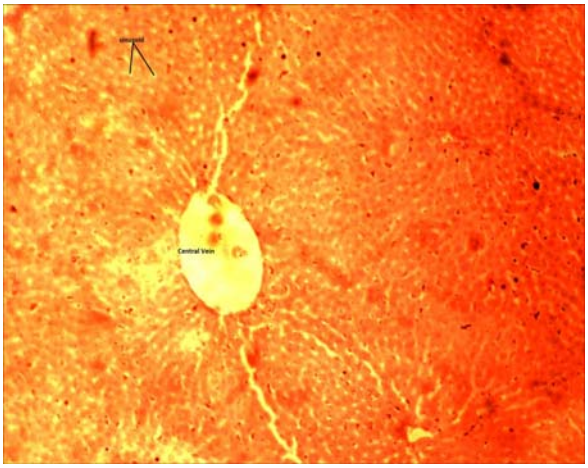

Plate 4: Test 3 (Control treated group): Normal sinusoid, with dilated central veins.

4. Discussion

Diabetes mellitus which is characterized by hyperglycemia was confirmed in test groups 1 and 2 (diabetic untreated group and diabetic treated group) 48 hours after injecting 100 mg/kg alloxan. In our previous study, *Aloe vera* gel administered at 0.4ml/100g per oral route significantly reversed the increased fasting blood glucose concentration in the treated group [5]. This confirms the beneficial effect of *Aloe vera* gel in managing hyperglycemia in T1DM.

Elevated serum AST and ALT are common in diabetics [13]. Among the various liver enzymes measured in this study, AST significantly increased (P<0.001) in test 1, compared with control. Although test 2 showed a decrease in AST concentration when compared with test 1, the decrease was not significant, suggesting that *Aloe vera* gel did not completely reverse the increased AST concentration. Animals in test 3 had significantly increased (P<0.001) AST concentration, compared with control, test 1 and 2. Serum ALT and ALP concentrations were significantly reduced (P<0.001) in test 1, compared with control. *Aloe vera* gel administered to test 2 resulted in an increase in ALT and ALP concentrations to levels of that of the control group, (Fig 2 and 3). Serum ALT concentration was significantly higher (P<0.001) in test 3, compared with control, while

ALP concentration in test 3 was not significantly different from control, (Fig. 2 and 3).

Ha *et al* [14] had reported liver damage secondary to *Aloe vera* gel ingestion. Surprisingly, the liver histology for test 3 (plate 4) in our study did not show defects as serious as that of the DM untreated group (plate 2), suggesting that *Aloe vera* gel may be increasing serum AST and ALT concentrations through a mechanism other than liver damage. It is established that AST and ALT maybe increased as body weight increases (as in obesity), and this increase may not necessarily depict liver damage. Nna *et al* [15] had earlier reported that *Aloe vera* gel increased body weight in normal animals without increasing food intake. The increased serum AST and ALT observed in test 3 in this study may be related to the effect of *Aloe vera* gel on body weight [15], rather than liver damage.

Serum proteins are important determinants of the oncortic property of blood. Albumin, which is synthesized mainly in the liver, constitute about two third of the total protein in serum and is responsible for transport of various materials including drugs in circulation. Serum total protein, albumin and globulin were significantly ($P<0.001$) reduced in test 1, 2 and 3, compared with control. Serum globulin concentration was significantly reduced ($P<0.05$) in test 2, compared with test 1. The low levels of proteins observed in the diabetic untreated group (test 1) in this study may be attributed to decrease synthesis of proteins and increase in the activities of gluconeogenesis, which is always at its peak in diabetics [6].

Considering the fact that the histology of the liver in test group 3 did not show marked defect, it is difficult to deduce that the observed reduced serum protein concentrations is directly linked to reduced protein synthesis. It is likely that protein was excessively excreted in the *Aloe vera* gel administered group.

5. Conclusion

Aloe vera gel mimics T1DM in increasing serum concentrations of liver enzymes, notably, AST and ALT, and reduces serum protein concentrations through a mechanism that may not be related to liver damage. Further research is necessary to investigate the mechanism through which this effect of *Aloe vera* is mediated.

6. Acknowledgement

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7. Competing Interest

None declared

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