Blood Sugar and histological Changes Following Administration of Ethanolic Leaf Extract of *Cassia alata* on Streptozocin-Induced Diabetic Albino Rats.

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ABSTRACT

The mechanism of most herbs used in the management of diabetes mellitus has not been well defined. This study was performed to investigate the hypoglycemic effects of ethanol leaf extracts of *Cassia alata* (candle bush) and its effect on the histology and glycogen content of the liver of streptozocin-induced diabetic albino rats. Twenty four (24) albino rats were used and were divided equally into six(6) groups; A, B, C, D, E, and F. Diabetes was induced in B, C, and D while A, E, and F were non diabetic. A, served as normal control while B served as diabetic control.

The two control groups received normal diet and was administered 0.3ml normal saline. C and E received 300mg/kg body weight of plant extract while D and F received 500mg/kg body weight of plant extract, given in two divided doses daily, 12 hours duration for twenty-one days. Blood glucose was measured every 7 days. At the end of the 21st day, the animals were anaesthetized and dissected. The livers were isolated, processed and stained with hematoxylin and eosin (H&E) and Periodic Acid Schiff's (PAS) staining techniques. The result of this study indicates that ethanol leaf extracts of *Cassia alata* was able to reduce blood sugar significantly in D (3.86±0.34) compared with B (20.8±5.86). And also in F (2.22±0.6) compared with A (4.17±0.33) using t-test ($P<0.05$). Increase in the density of liver glycogen was obvious in the groups that received 500mg/kg body weight of extracts. Leaf extracts of *Cassia alata* has dose-dependent hypoglycemic effects and increases the glycogen content of the liver though the mechanism of action is yet to be determined.

(Keywords: *Cassia alata*, diabetes mellitus, liver)

INTRODUCTION

Diabetes mellitus is a serious metabolic disorder with micro and macro vascular complications that results in significant morbidity and mortality [Rang *et al., 1991*]. The increasing number of ageing population, consumption of calorie- rich diet, obesity and sedentary life style have led to a tremendous increase in the number of diabetics worldwide. According to the world health organization, the prevalence of diabetes is most likely to increase by 35% from about 171 million people worldwide in year 2000 to about 300 million by the year 2030 [Wild *et al*., 2004].

The greatest increases seem to occur in developing countries like Nigeria and India because it follows the trend of urbanization and changes in life style. Despite efforts to control it, diabetes remains an undefeated enemy in its battle with orthodox medicine.

Herbal medicine in many respects has gained a new momentum in the medical field. In developing countries like Nigeria particularly in rural areas and poor urban areas, herbal medicine is in most cases, the only form of health care that sick persons immediately turn to and only as a last resort will they consult a regular physician. The traditional use of plants in the treatment of various infections and illnesses is practiced in most parts of Nigeria and this is in consonance with the WHO's recommendation that this should be encouraged where access to the conventional treatment is not adequate [WHO 1980].

Cassia alata is a shrub from the leguminosae family employed in traditional medicine for the treatment of various ailments [Dymock, 1980]. It grows in tropical region of Nigeria and can also be found in other countries [Ibrahim et al., 1995].

Its common name is "*bush candle*" because of the orientation of the flowers like the shape of a candle flame but it is more popularly called Ringworm plant because it is known to be very effective in the treatment of ringworm infection. In an ethno-botanical survey which identified and documented twenty-two [22] plants from south-western Nigeria used by traditional healers in the region, *Cassia alata*, closely followed by *Vernonia amygdalina del* remains the most frequently used plant in the management of diabetes [Abo et al., 2000].

Figure 1: *Cassia alat.*

MATERIALS AND METHODS

Plant Material

Fresh matured leaves of *Cassia alata* were harvested, rinsed and dried under shade at room temperature (28±30^{0}C). The dried leaves were ground with a manual grinding machine and sieved. 570g of the fine powder was soaked in 2500ml of ethanol (80%) and mixed thoroughly for about 5 minutes with an electric blender. The suspension was poured into a plastic container and allowed to stand for 48 hours in a thermocool fridge at -4^{0}C.

After 48 hours, the mixture was filtered with a sifter and the filtrate gotten was re-filtered with a filter paper (Hartmann's no. 1) and left to stand overnight. The filtrate was concentrated in a Soxhlet extractor to about 20% of its original volume. The concentrate was allowed in a water bath at 60^{0}C for complete dryness yielding 25g of the crude extract which was then stored at -4^{0}C in a fridge. This extract was finally re-constituted in normal saline to an appropriate concentration before administration.

Animal Breeding

Twenty-four albino rats, male and female weighing between 180g to 200g was obtained**.** They were kept for acclimatization for a period of 2 weeks in the animal house at a temperature of 28±2^{0}C and relative humidity 50±5% with a 12 hour light/dark cycle.

The rats were housed in suitable wooden cages with netted covers and saw dust beddings which was regularly cleaned and replaced to maintain a suitable hygienic environment for the animals. The rats were fed with growers mash in small dishes and water through water bottle fitted with suction-controlled nozzles.

Induction of Diabetes

Diabetes mellitus was induced by single intra-peritoneal injection of 65mg/kg of streptozocin dissolved in normal saline into 12 hour fasted rats. After 96 hours of STZ-injection, the rats were fasted for 12 hours and capillary blood was taken from the tail artery of the rats. Diabetes was confirmed by determining the Random Blood Glucose (RBG) level (200mg/dl) using an automated glucose sensor machine – glucometer analyzer (One Touch Basic®). Polyuria was observed 72 hours later followed by polydipsia and polyphagia.

Experimental Design

The design consisted of six (6) groups of four rats each.

Group A: Normal control received 0.3ml normal saline.

Group B: Diabetic control received 0.3ml normal saline.

Group C: Diabetic treated group (low dose) received 300mg/kg body weight of herb extract.

Group D: Diabetic treated group (high dose) received 500mg/kg body weight of herb extract.

Group E: Non-diabetic treated group (low dose) received 300mg/kg body weight of herb extract.

Group F: Non-diabetic treated group (high dose) received 500mg/kg body weight of herb extract.

At the end of the study, the animals were anaesthetized under chloroform vapor and the abdomen dissected to remove the liver. The liver tissues were fixed in Bouin's fluid. The tissues were prepared using routine histological technique and stained with hematoxylin and eosin (H&E) and Periodic Acid Schiff (PAS) technique.

RESULTS AND DISCUSSION

The following observations were made:

Table 1: The Effect of Herb Extract on the Mean Weight of the Animals During the Course of the Study.

Group	A	B	C	D	E	F
Weight before Administration	180 ±8.16	180 ±2.5	180 ±7.1	180 ±2.88	200 ±4.5	200 ±0.1
Weight after Administration	192.5 ± 9.8	125 ±4.1	131.75 ±7.1	141.5 ±5.0	162.5 ±8.7	147.5 ±2.88

Values are mean ± SEM

The effect of leaf extract on mean weight of animals after the period of administration is shown in table 1. The mean weight of the group A increased from 180±8.16 to 192±9.8 while that of group B (diabetic control) animals dropped remarkably from 180±2.5 to 125±4.1 during the period.

In comparison with B, group C (low dose) animals showed a higher mean weight of 131.75±7.1 after administration while group D (high dose) animals showed a higher mean weight of 141.5±5.1 after administration. However, a significant reduction in weight was observed in all the groups that received the extract.

Groups E and F showed a significant decrease in body weight relative A. Group D animals also showed a significant increase in body weight relative to B using t-test at $P<0.05$.

Table 2: The Effects of Herb Extracts on the Mean Fasting Blood Glucose (FBG) During the Period of Study.

Group	A	B	C	D	E	F
FBG before Administration	4.25 ±0.5	21.7 ±7.3	20.75 ±7.53	21.1 ±7.41	4.58 ±2.6	4.2 ±0.4
FBG after Administration	4.17 ±0.33	20.8 ±5.86	17.62 ±5.0	3.86 ±0.34	3.83 ±0.53	2.22 ±0.6

Values are mean ± SEM

The effect of the leaf extract on mean FBG is presented in table 2. The mean FBG values in diabetic untreated group (diabetic control) and non-diabetic untreated group (normal control) was 20.8±5.86 and 4.17±0.33, respectively. Comparing the diabetic untreated with the diabetic treated groups, the groups that consumed the extract showed lower mean FBG that is 17.62±5.0(Group C) and 3.86±0.3(Group D) for low and high dose groups, respectively.

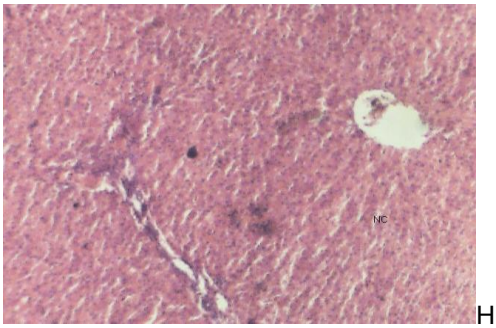
In comparison with the non-diabetic untreated (normal control) group, the non-diabetic groups (E and F) that consumed the extract also showed relatively lower FBG levels of 3.83±0.53 and 2.22±0.6 for low and high dose groups, respectively.

The observations are presented as mean ±SD using $P<0.05$ and student t-test. The groups that consumed the extract at 500mg/kg showed a significant decrease in blood glucose while the groups that consumed the extract at 300mg/kg showed a relative decrease in the blood glucose also which is not significant using the t-test.

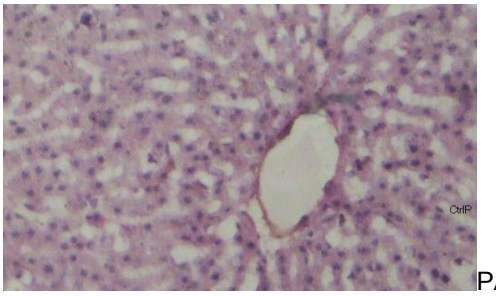
Besides the remarkable changes that occurred in the mean FBG and mean weights, sluggishness was observed in the diabetic groups. Polyuria accompanied by polydipsia was observed in groups B, C and D. However, after the first 10 days of extract administration, the symptoms were ameliorated in group D animals.

HISTOLOGICAL OBSERVATIONS

Liver section of experimental animals stained with hematoxylin and eosin (H and E) method and Periodic Acid-Schiff's technique are displayed in the plates below showing distinct features and relative differences between the control and treated groups.



H & E

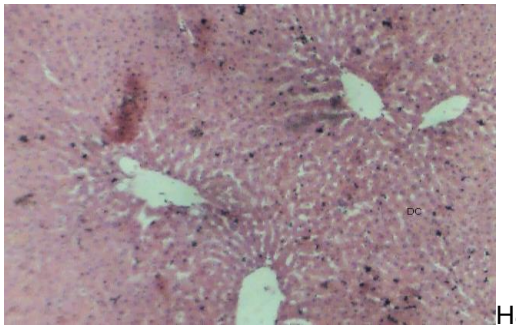


PAS

Plate 1: A and B (Group A – Normal Saline)

It shows the liver lobule and includes a number of hepatic plates. The polygonal boundary of the lobule is partly defined by two portal tracts and sparse collagenous tissues. The sinusoids that originate from the margin of lobule are seen to course between plates of hepatocytes to converge at the central vein. The hepatocytes are one-cell thick in some places and two to three cell thick in other places. The sinusoids are lined by endothelium that accommodates other macrophages called Kupffer cells.

The PAS stained section reveals a significant quantity of glycogen within the cytoplasm of hepatocytes.



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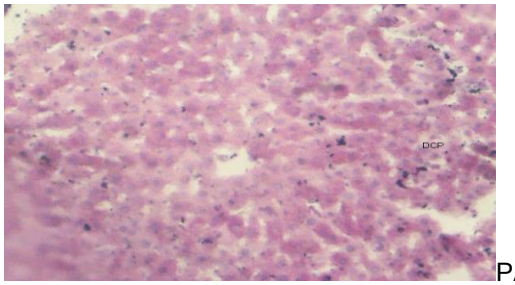
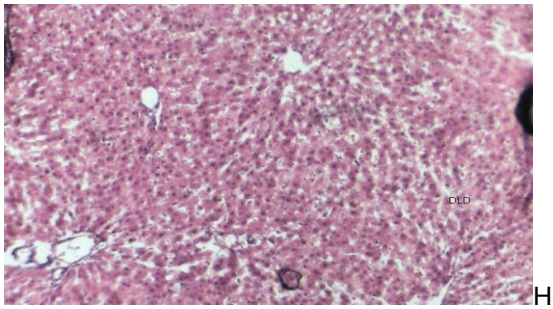


PAS

Figure 2: A and B (Group B – Normal Saline)

Micrograph shows that several central veins have reduced diameter. There are numerous vacuoles in the liver parenchyma and within the hepatocytes. There is hypertrophy of hepatocytes and the nuclei are displaced to the rim of cytoplasm by the vacuole. PAS stained section shows reduced magenta-stained glycogen granules.



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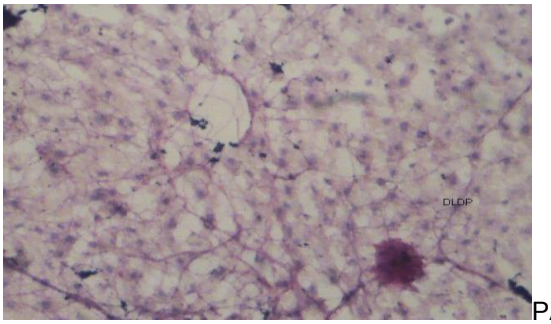
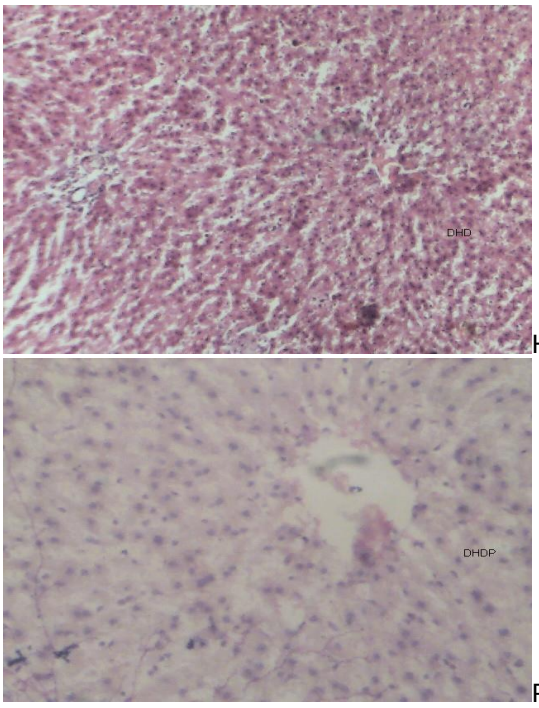


PAS

Plate 3: A and B (Group C–300mg/kg of extract).

This photomicrograph shows portal tracts at strategic locations with hepatocytes plates and sinusoids radiating towards the central vein. There is stenosis of the sinusoids in some regions and complete blockage in other regions. Numerous vacuoles are observed. PAS sections also show enlarged numerous vacuoles. Glycogen content is reduced.



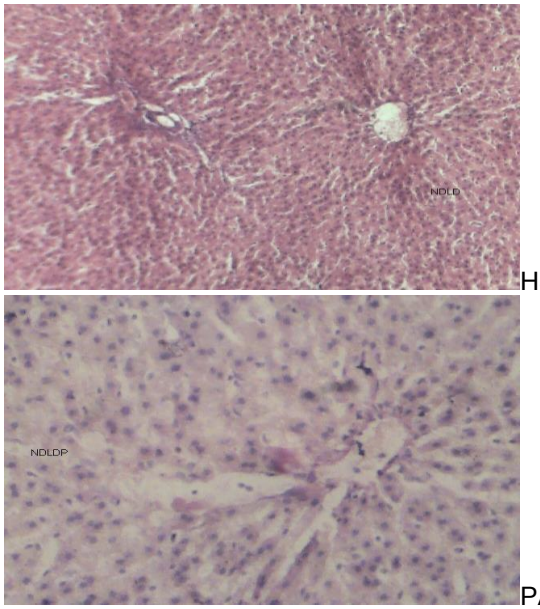
H&E

PAS

Plate 4: A and B (Group D – 500mg/kg of extract).

This photomicrograph shows portal triad enmeshed in a fibrous stroma. Simple cuboidal epithelium lines the bile duct. There are numerous Kupffer cells which lines the sinusoids. Anastomosing plates of hepatocytes are mostly two or three cells.

With PAS, the intra-cytoplasmic environment of hepatocytes shows numerous PAS – positive glycogen granules. Elastic fibres also run across the section. The vacuoles are greatly reduced when compared with group B (fig 2.2) and C (fig 2.3) section.

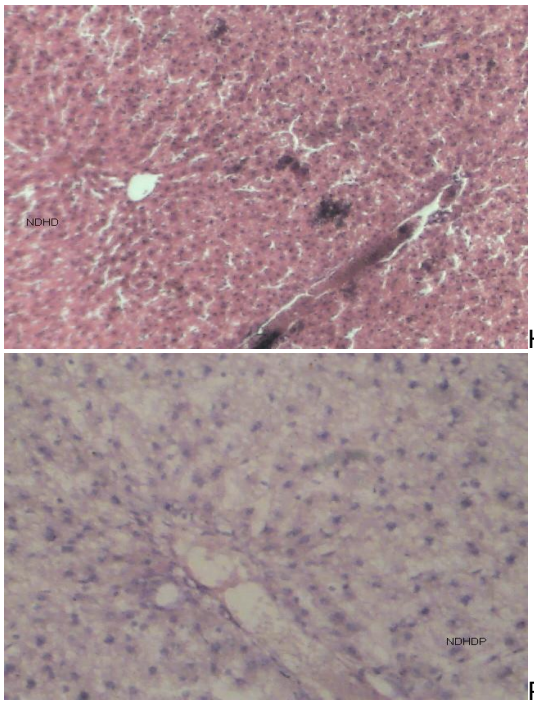


H&E

PAS

Plate 5: A and B (Group E – 300mg/kg of extract).

The micrograph shows a portal tract and the central vein. The orientation of portal triad, hepatic plates and sinusoids are similar to that of group A. PAS technique shows numerous glycogen granules.



H&E

PAS

Plate 6: A and B (Group F - 500mg/kg of extract).

Micrograph with H&E shows portal tract and central vein. There is increase in number of hepatocytes on each hepatic plate. There is regeneration and ballooning of hepatocytes.
PAS technique shows marked increase in the intra-cytoplasmic glycogen granules that are distributed throughout the section.

DISCUSSION

Many traditional plant treatments for diabetes mellitus are used throughout the world (Bailey and Day, 1989), and particularly in Africa. Plants may act on blood glucose through different mechanism, some may have insulin – like substances, some may inhibit insulinase activity, others may cause increase in pancreatic β-cells by activating regeneration of these cells (Abdel et al., 1997). The fibres of plants may also interfere with carbohydrate absorption, thereby affecting blood glucose (Nelson et al., 1991).

The high level of fasting blood glucose and cellular damage observed in diabetic animals (group B, C and D) was as a result of oxygen free radicals formed by the presence of streptozocin which also caused the accumulation of TBARS which led to hepatocyte degeneration (Ohkuwa et. al., 1995).

The effect of leaf extract of *Cassia alata* carried out in this work showed a dose- dependent decrease in the fasting blood sugar significant at $P<0.05$ using students t-test. The reduced blood glucose noted indicates that leaf extract of *Cassia alata* possess chemical components that have hypoglycemic properties and this potency might be the primary reason why it is the most frequently used plant in the management of diabetes mellitus in south-western Nigeria(Abo et al., 2000).

A significant hypoglycemic effect was also observed in group F animals from 4.15±0.4 to 2.22±0.6. This can be attributed to the effects of several bioactive components like Naringenin, Quercetin and Luteolin. Studies by Nica, Murray et al (2003), showed that Naringenin demonstates insulin- like effects in vivo. Luteolin is a potent hypoglycemic agent which improves insulin sensitivity. It is also used in weight management (Menon et al., 2004). Studies with Quercetin on STZ – induced diabetic animals also resulted in lowering of blood glucose and ameliorated diabetes –induced oxidative stress (Menon et al., 2004). Kaempferol and Quercetin have been shown to have anti-inflammatory effects on the liver (Garcia et al, 2008), protects against oxidative stress and lowers plasma TBARS (Menon et al., 2004).

The general decrease in weight in all animals that consumed the extract at both 300mg/kg and 500mg/kg can also be attributed to the bioactive components in *Cassia alata*. Recent studies by Christine et al (2011) revealed that *Cassia fistula* and *Senna alata* significantly and effectively reduced the body weight of mice due to their tannin content and that both plants are potential sources of anti - obesity and hypolipidemic compounds.

Histological studies of liver sections showed marked differences between the various groups. Decreased glycogen content observed in group B and C suggests that at 300mg/kg, the diabetic symptoms were not controlled. In group D, however, the presence of significant quantity of glycogen granules, reduced vacuoles, and multiplication of Kupffer cells and regeneration of hepatocytes indicates corrective effect of the herb on the diabetic status. The group E sections did not show any significant difference compared to A. Marked increase in glycogen content in group F (500mg/kg body weight) is due to effect of Quercetin. Quercetin is also known to increase liver glycogen content, normalizes glycaemia and decreases cholesterol and low density lipoprotein in diabetic patients (Nuraliev and Avezor, 1992). Regeneration and ballooning of hepatocytes is probably due to increased macromolecule – uptake effect of the herb. The findings support traditional use of *Cassia alata* for controlling hyperglycemia in diabetics (Abo et al., 2000).

SUMMARY AND CONCLUSION

Having noted the various effects of leaf-extract of *Cassia alata* on blood glucose, histology and glycogen content of the liver as shown with a light microscope, it is obvious that it is anti-hyperglycaemic and non-toxic to the liver. Based on the result obtained from this work, significant changes was not observed at a low dose of 300mg/kg but a very remarkable improvement was observed at the dose of 500mg/kg. This shows that the effects of hydro-ethanol leaf extract of *Cassia alata* is dose dependent.

It can therefore be concluded that hydro-ethanol leaf extract of *Cassia alata* has hypoglycemic effects, increases the glycogen content of the liver and generally improves liver cytoarchitecture.

RECOMMENDATIONS

This work is recommended for further studies on other organs like kidney, pancreas and other effects like antiviral effects.

Electron microscopy should be employed in further studies to reveal the effects at molecular level.

The effects of this herb should also be tested for a longer duration to ascertain its long-term effects.

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