Effects of Hydromethanolic Extract of Cnidoscolus Aconitifolius *(Buphorbiacaea)* on Body Weight, Some Liver Enzymes and Histology in Diabetic Wistar Rats

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***Abstract*-This study evaluated the effect of hydromethanolic leaf extract of Cnidoscolus Aconitifolius (C.A) (euphorbiaceae) on body weight, some liver enzymes and histology in Streptozotocin (STZ) induced diabetic Wistar rats. Thirty-six wistar rats of both sexes with an average weight of 230g were divided into six groups of 6 rats each. The rats were fasted for 12 hours and diabetes induced intraperitoneally using 60mg/kg-l b.w of STZ. Group l: Untreated non-diabetic, Group 2 received 10mg of glibenclamide, Group 3: Untreated diabetic, served as the positive control. Groups 4, 5 and 6 were treated with 100, 150 and 200 mg/kg b.w of the extract respectively. On day 29, the animals were anesthetized using chloroform and sacrificed. The body weight of animals was determined using an electric weighting balance and blood samples collected by cardiac puncture for the determination of some liver enzymes, while the liver were collected and fixed in 10% formalin for histological studies. Results show that the extract significantly increase the body weight of experimental animals in a dose dependent manner in the test groups ($p<0.05$). There was also a significant increase in liver enzymes in groups 4, 5 and 6 compared to Groups 2 and 3 ($p<0.05$). Histological studies show some levels of degenerations in the tissues of the liver. Result suggest that C.A could encourage weight gain but have adverse effect on the liver as seen in the elevated serum liver enzymes supported by the histological studies.**

Key words: **Cnidoscolus Aconitifolius, Streptozotocin, diabetes, hydromethanolic, chloroform.**

I. INTRODUCTION

Weight gain is an increase in body weight: this can involve an increase in muscle mass, fat deposits, excess fluids such as water or other factors [1]. Maintaining a healthy weight is important for health. In addition to lowering the risk of heart disease, stroke, diabetes, and high blood pressure, it can also lower the risk of many different cancers [2].

Diabetes mellitus is fast emerging as a health challenge globally threatening to reach pandemic level by the year 2030 due to poor dietary habits, sedentary life style and increasing prevalence of obesity [3]. In developing countries, the increased incidence of diabetes has been ascribed to dietary and lifestyle changes with the associated shift from the relatively healthy traditional lifestyle to an unhealthy lifestyle [4]. This condition has been reported to have adverse effects on the blood glucose, body weight, lipid distribution and some liver enzymes of affected individuals.

Liver disease may occur as a result of diabetes and the reverse is true as well. The liver plays a role in glucose regulation: glucose is transported from the intestine to the liver which stores it as glycogen or uses it for fuel [5]. Insulin regulates the movement of glucose into tissues and promotes glycogen storage; insulin is metabolized in the liver, where it promotes the production of glycogen, protein, cholesterol and triglycerides and stimulates the formation of low density lipoprotein which transports cholesterol into arteries [6]. In diabetes excessive output of glucose by the liver contributes to elevated fasting blood sugars. Fat accumulation in the liver may be linked to excess glycogen which is common among diabetics. Fatty deposits may be due to the increase transport of fats to the liver from the intestines, the condition occurs secondly to obesity as well as diabetes, but the exact reason is unknown [7]. Liver function test (LFTs), which includes liver enzymes are group of chemical biochemistry laboratory blood assays designed to give information about the patient's liver [8]. Most liver diseases cause only mild symptoms initially, but it is vital that these diseases be detected early. Hepatic (liver) involvement in some disease can be of crucial importance. These liver function tests are used to: distinguish among different types of liver disorders, detect the presence of liver disease, follow the response of the treatment and gouge the extent of known liver damage [9].

Cnidoscolus aconitifolius commonly known as chaiya or tree spinach, is a perennial shrub belonging to the family Euphorbiaceae, is easy to grow, a tender perennial in the U.S, and suffers little insect damage: Propagation is normally by wooden stem cuttings about 6-12inches long. The edible parts of the plant, which taste like Spinach when cooked, serve as important nutritional source of protein, vitamin and minerals especially for populations that cannot afford expensive foods

rich in these nutrients [10]. Cnidoscolus aconitifolius shoots and leaves have been taken as laxative, heretic and circulating stimulant to improve diabetes, digestion, stimulate lactation and harden fingernails. They have high fiber content and antibacterial activities which have been reported by various authors including Awoyinka *et al*., (2007) [11]. Reports on the possible effect of the hydromethanolic Leaf Extract of Cnidoscolus Aconitifolius (Euphorbiacaea) on body weight, some liver enzymes and histology in diabetic rats have been scanty and this justifies our study. The present study therefore, presents a preliminary report of the effect of hydromethanolic Leaf Extract of Cnidoscolus Aconitifolius (Euphorbiacaea) on body weight, some liver enzymes and histology in diabetic rats.

II. MATERIALS AND METHODS

This study was carried out in the animal house of the Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, Choba, Nigeria for a period of 28 days.

A. Plant materials and extraction preparation

Fresh leaves of Cnidoscolus aconitifolius were ploughed from a local garden at Seventh Day Adventist Church, Choba, Port Harcourt, Rivers State and were identified by Dr. N. E. Edwin-Wosu of the department of Plant Science and Biotechnology, College of Natural and Applied Sciences, University of Port Harcourt, Choba, Rivers State, Nigeria. Voucher specimen of the plant was deposited in the herbarium with the reference number: UPH/PSB/015. The research protocol for the study was approved by the Ethics Committee of our institution. This study was conducted in accordance with the guidelines for the care and use of laboratory animals issued by the United States Institute for Laboratory and Animal Research (1996) [12].

Fresh leaves of Cnidoscolus aconitifolius were air dried for a minimum of 14 days and extracted using the percolation method. The dried leaves were pulverized with electric grinding machine into minute pieces. Hydromethanolic (1/4, v/v) extraction was carried out with soxhlet extractor (model no. 3567, Austria). The chloroform fraction of the extract was obtained and filtered using Whatman No 1 filter paper. The filtrate was concentrated under reduced pressure in vacuum at 45°C using rotator evaporator (GallenKamp UK). The resulting residues called dried leaf extracts were transferred to a hot oven where they were dried to a constant weight at 45°C. The extract was stored at 4°C.

B. Experimental animals and drugs

This study was carried out using 36 (thirty six) matured wistar rats, weighting between 220-280g. The animals were kept at the Animal House, Department of Physiology, Faculty of Basic Medical Sciences, University of Port Harcourt, Nigeria, with a 12 hour light/12 hour dark cycle. The rats were fed with normal rat pellet and tap water *ad libitum*. The experimental animals were acclimatised for a period of two weeks after which they were grouped. Streptozotocin was acquired from Sigma-Aldrich Co., 3050 Spruce Street, St. Louis, USA. While glibenclamide was obtained from Swiss Pharm Nigeria Ltd, 5, Dopemu Road, Agege, Lagos, Nigeria.

C. *Experimental design*

Thirty six wistar rats of both sexes, with average weight of 230g, were randomly assigned into six groups (n=6). Diabetes was induced after an overnight fast through a single intraperitoneal injection of 60mg/kg body weight of streptozotocin dissolved in distilled water. Diabetes was confirmed after 72 hours of *streptozotocin* administration if the blood glucose is ≥ 190mg/dl [13]. Each animal group was treated as follows:

Group 1: Untreated non-diabetic: rats in this group received only normal rat chow and tap water *ad libitum*.

Group 2: diabetic + glibenclamide; rats in this group were treated with 10mg/kg b.w of glibenclamide after the induction of diabetes.

Group 3: Untreated diabetic: rats in this group received no further treatment after induction of diabetes.

Group 4: diabetic + low dose *Cnidoscolus Aconitifolius;* rats in this group were treated with 100mg/kg b.w of *Cnidoscolus Aconitifolius* extract once in two days after the induction of diabetes.

Group 5: diabetic + medium dose *Cnidoscolus Aconitifolius;* rats in this group were treated with 150mg/kg b.w of *Cnidoscolus Aconitifolius* extract once in two days after the induction of diabetes.

Group 6: diabetic + high dose *Cnidoscolus Aconitifolius;* rats in this group were treated with 200mg/kg b.w of *Cnidoscolus Aconitifolius* extract once in two days after the induction of diabetes.

D. Determination of body weight and liver enzymes

Body weight of rats was determined using the method described by Yaghoobi *et al.,* 2008 [9]. During the course of the study, body weight of experimental animals was determined thrice: firstly, prior to administration of streptozotocin to induce diabetes (Day 0); secondly, 72 hours after administration of streptozotocin (Day 3); and thirdly, at the end of study (Day 29). Liver enzymes were also estimated at the end of study (Day 29) using the method described by Wallach, 2007 [8].

E. Statistical analysis

Results for body weight and liver enzymes are presented in figures expressed as means ± standard error of means. Significant differences were determined using the one-way ANOVA. A p value of less than 0.05 was considered statistically significant

III. RESULTS

A. Effects of hydromethanolic extract of Cnidoscolus Aconitifolius (Buphorbiacaea) on body weight

Figure 1 shows the effects of hydromethanolic extract of Cnidoscolus Aconitifolius *(Buphorbiacaea)* on body weight in diabetic Wistar rats. Compared to Group 3 (untreated diabetic) rats, administration of extracts of Cnidoscolus Aconitifolius significantly increase the body weight of experimental animals in a dose dependent manner amongst Groups 4, 5 and 6 rats ($p< 0.05$); suggesting a positive effect of the extract on body weight in diabetic animals. This effect of Cnidoscolus Aconitifolius is comparable to the effect of administration of glibenclamide observed amongst Group 2 rats. At a dose of 200mg/kg b.w amongst Group 6 rats, the percentage weight change was marginally higher than that observed for glibenclamide amongst Group 2 rats. Perhaps suggesting a greater potency of hydromethanolic extract of Cnidoscolus Aconitifolius compared to glibenclamide.

B. Effects of hydromethanolic extract of Cnidoscolus Aconitifolius (Buphorbiacaea) *on some liver enzymes*

Figure 2 and 3 shows the effects of hydromethanolic extract of Cnidoscolus Aconitifolius *(Buphorbiacaea)* on some liver enzymes. Compared to Group 3 (untreated diabetic) rats, administration of extracts of Cnidoscolus Aconitifolius significantly increase the serum concentration of AST, ALT and ALP of experimental animals in a dose dependent manner amongst Groups 4, 5 and 6 rats ($p< 0.05$); suggesting a possible toxic effect of the extract on the liver of diabetic animals on prolong exposure. This effect of Cnidoscolus Aconitifolius is comparable to the effect of administration of glibenclamide observed amongst Group 2 rats. However, the effect of glibenclamide administration amongst Group 2 rats suggests a greater potency compared to the extract administration on liver enzymes alone.

C. *Histologic changes in the liver epithelium*

Plate A is a cross section of the liver epithelium obtained from Group 1 (untreated non-diabetic) rats showing intact and tightly arranged hepatocytes. Plate B is a cross section of the liver epithelium obtained from Group 2 (diabetic + glibenclamide) rats showing degeneration of hepatocytes. Plate C is a cross section of the liver epithelium obtained from Group 3 (untreated diabetic) rats showing severe degeneration and portal vein filled with inflammatory cells. Plate D is a cross section of liver epithelium obtained from Group 6 (2oomg/kg b.w of C.A) rats showing severe balooning and degeneration of hepatocytes.

IV. DISCUSSION

The present study essentially determined the effects of hydromethanolic extract of Cnidoscolus Aconitifolius *(Buphorbiacaea)* on Body weight, some Liver enzymes and histology in diabetic Wistar rats. There is significant increase in weight of animals treated with the extract relative to the control; this may be due to the presence of metabolites as alkaloids. This corroborates the report of [14] that alkaloids stimulate protein synthesis and weight gain. The results obtained indicating an increase in body weight of treated animals clearly suggest that the extract of cnidoscolus aconitifolius apparently improves weight gain in streptozotocin induced diabetes in a dose dependent manner; comparable to the effect of glibenclamide a known anti-diabetic agent used commonly in the management of diabetes.

The findings of this study also suggest that the extract effect when subjected to liver function test shows significant ($p<0.05$) increase in the liver AST, ALT and ALP of the hydromethanolic extract of cnidoscolus aconitifolius treated rats, when compared to the diabetic control group. It is known that an increase in the enzymatic activity of ALT, AST and ALP in the serum directly reflects a major permeability or cell rupture (Benjamin 1978). The transaminases (AST and ALT) are often used as specific markers of active hepatic injury and represent markers of hepatocellular necroses [15]. These liver enzymes catalyses the transfer of a-amino groups of aspartate and alanine to a-keto group of a-Ketoglutaric acid [15]. AST activity is present in a wide variety of tissues including heart, skeletal muscles, kidney, brain and liver, whereas ALT activity is primarily localized in the liver and largely specific for parenchymal disease [16]. The serum levels of ALT and AST are usually increased during hepatitis [16]. Therefore significant increase in AST, ALT and ALP levels suggest a toxic effect of the extract in the liver on prolonged exposure.

The histologic studies show that most of the organs where balooted. The liver of group 1 rats show normal hepatic cells, while those that received C.A show some alterations in the liver cells.

V. CONCLUSION

In conclusion, the results of the present study show that the hydromethanolic extract of Cnidoscolus Aconitifolius causes weight gain and a balooning degeneration of hepatocytes in the liver on prolong exposure following streptozotocin induced diabetes in wistar rats; these findings along with the histological changes described suggests a possible beneficial effect of the extract on body weight and a potential toxic effect on the liver of experimental animals. The results indicate a possible beneficial effect of the extract and perhaps a therapeutic potential in weight management. We recommend further studies in this regard.

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LEGEND TO FIGURES

Figure 1: shows weight change of rats before and after extract treatment
Figure 2: shows serum concentration of liver AST and ALT following treatment
Figure 3: shows serum concentration of ALP following treatment

LEGEND TO PLATES

Plate A: shows the liver histology of untreated non-diabetic rats.
Plate B: shows the liver histology of diabetic + glibenclamide rats.
Plate C: shows the liver histology of untreated diabetic rats
Plate D: shows the liver histology of diabetic + high dose C.A rats

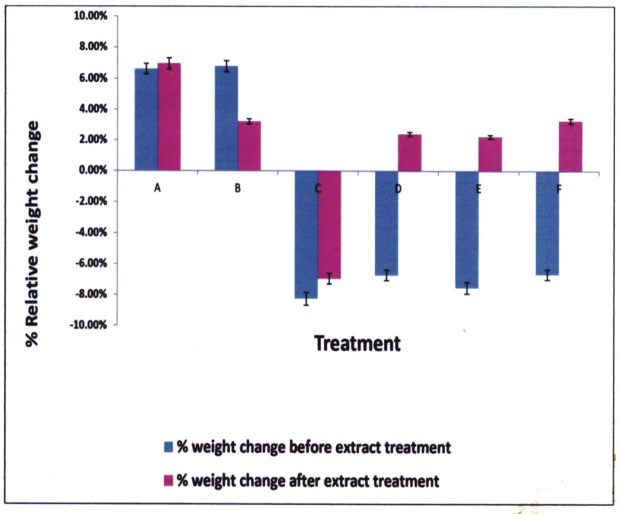

Fig 1:

KEY
A= (untreated non-diabetic)
B= (diabetic + glibenclamide)
C= (untreated diabetic)
D= (diabetic + low dose C.A)
E= (diabetic + medium dose C.A)
F= (diabetic + high dose C.A)

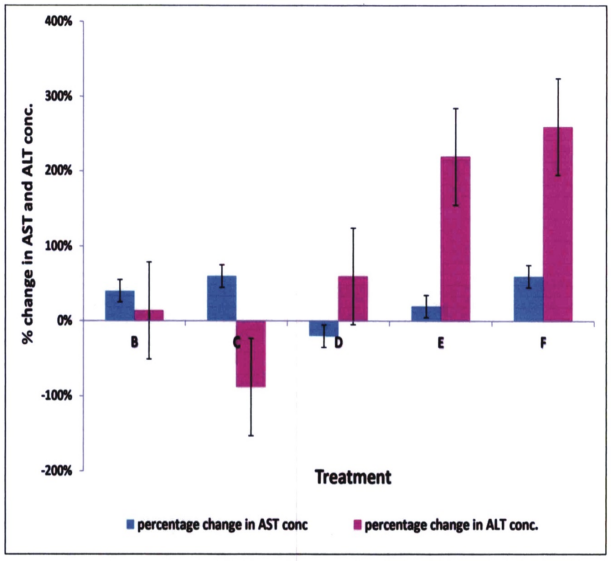

Fig 2:

KEY

A= (untreated non-diabetic)
B= (diabetic + glibenclamide)
C= (untreated diabetic)
D= (diabetic + low dose C.A)
E= (diabetic + medium dose C.A)
F= (diabetic + high dose C.A)

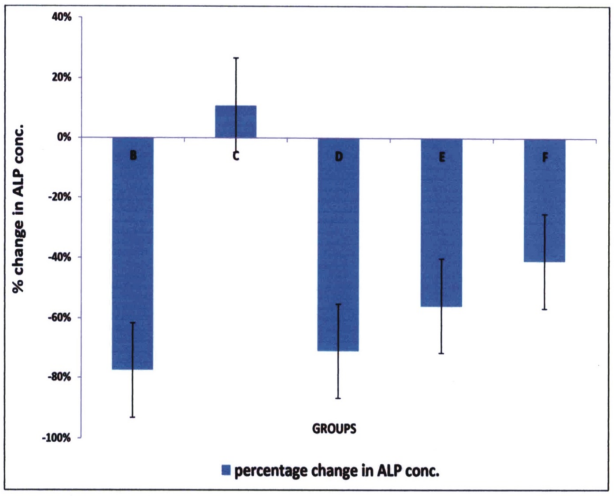

Fig 3:

KEY

A= (untreated non-diabetic)

B= (diabetic + glibenclamide)

C= (untreated diabetic)

D= (diabetic + low dose C.A)

E= (diabetic + medium dose C.A)

F= (diabetic + high dose C.A)

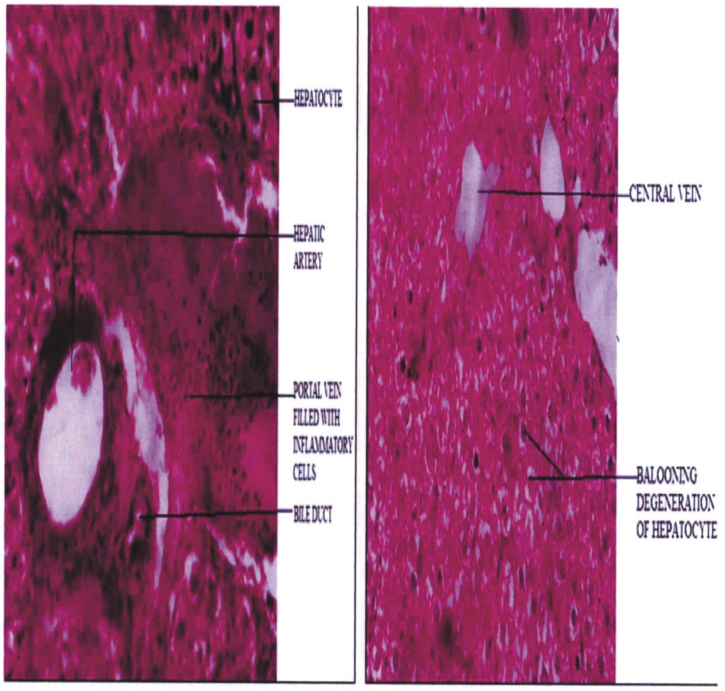

Plate C: Plate D:

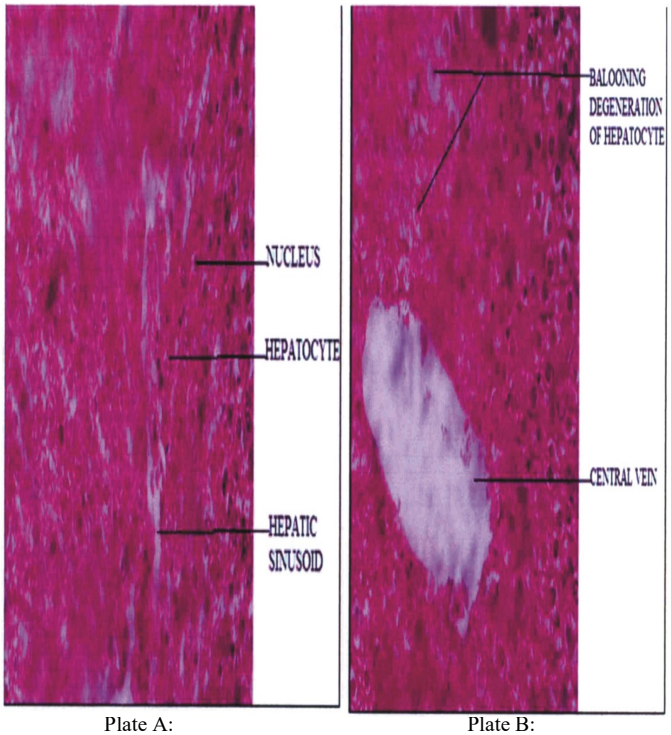

Plate A: Plate B: