Effect of Extracts of *Diaphananthe bidens* Leaves on Organ Pathogenesis and Histopathology of Diabetic Rats

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Abstract

Twenty (20) male wistar rats of average body weight 160g were divided into groups of ten (10) each. One group was made diabetic with alloxan while the other group remained non-diabetic. Each group was treated with crude ethanol and water extracts of the leaves of Diaphananthe bidens for seven (7) days and the effect of this on the organs;- kidney and liver were investigated. Another set of 20 male wistar rats of similar weight were divided into groups of ten (10) each. One group was made diabetic with alloxan and treated with glibendamide and the other group received normal saline with no treatment; these groups served as the control. All the rats were maintained ad libitum on chick's mash and water and were sacrificed after 7 days. The result showed a significant reduction in body weight gain (P<0.05) relative to control rats. None of the visceral organs showed a significant increase (P>0.05) in weight when compared with their control. Histological changes were however observed in the kidneys and liver of animals treated with the extracts. These changes were characteristically vascular, degenerative /necrotic and inflammatory. These results suggest that the crude ethanol and water extracts of the leaves of Diaphananthe bidens may precipitate mild organ damage.

Keywords: *Diaphananthe bidens* extract, Rat, Diabetes, Histopathology

Introduction

Diaphananthe bidens [(Afzel. ex.sw.) schltre, 1914] (Orchidaceae) is an epiphyte also commonly known as "two toothed Diaphananthe" is distributed within the tropical zones of Africa. It grows at an elevation of 350 to 1300 m above sea level as a medium sized plant. It has an elongated stem carrying numerous ovate, unequal bilobed leaves that bloom in the dry season (Guy, 2004). *Diaphananthe bidens* is known to have no food value associated with any part of it. Although documented information on the ethnomedicinal uses of this plant is scarce in literature, it is widely used in the treatment or management of *diabetes mellitus* in the Manyu Division of the South West Providence of Cameroon. Also, ethanol extract of the dried leaves is also widely used to manage diabetes in Nsukka, Enugu State, Nigeria. Here, about 25 ml of the extract is taken 8 hourly for seven days (Pers. Comm, 2007). Diabetes is a chronic disease that needs prompt institution of treatment or management to control blood sugar levels and prevent complications arising from multiple organ damage. Some of the complications may not have obvious symptoms and can permanently destroy such vital body organs as the eyes, kidneys and liver.

In order to assess the usefulness of this plant in the treatment of diabetes, this work was undertaken to evaluate the effect of its extracts on the liver and kidney of diabetic rats using histopathological examination of these organs in treated animals.

Materials and Methods

Plant materials: Fresh leaves of *D. bidens* were collected from the environs of Nsukka LGA, Enugu State, Nigeria and was identified by Mr. A. O. Ozioko of Bioresources Development and Conservational Programme (BDCP), Nsukka. The leaves were cut smaller pieces, dried at room temperature (25 °C) for seven days and pulverised to a coarse powder with mortar and pestle.

Extraction: The powdered plant material (500 g) was extracted by cold maceration in ethanol (1 L; analytical grade; BDH) or distilled water (1 L) for 24 h with occasional stirring. The extracts were filtered and concentrated in a fume chamber (ethanol extract) or regulated oven maintained at 60 °C (aqueous extract) to obtain 65 g of the ethanol extract and 45 g of aqueous extract respectively.

Animals: Adult albino rats (100-180 g) were obtained from the Animal House of the Faculty of Biological Sciences, University of Nigeria, Nsukka. The animals were maintained on standard pellets (Bendel Feeds Ltd., Nigeria) and tap water *ad libitum*.

Studies of the effect of the extracts on organs: The effect of the extracts on the kidney and liver of treated diabetic rats was evaluated. Animals were divided into 4 groups (n = 10/group). Groups I and II received 750 mg/kg of the aqueous extract and ethanol extract respectively. The Control groups (III and IV) received normal saline (0.85% w/v) and glibenclamide (600 mg/kg) respectively. Diabetes was induced by intraperitoneal injection of alloxan monohydrate (200 mg/kg; Sigma USA) in normal saline to normglycemaic rats having sugar concentrations of 50-74 mg/dl after 12 h fasting. After 7 days, the surviving rats were fasted for 12 h

and their blood sugar concentration determined using a glucometer. Animals with blood sugar concentration above 150 ml/dl were considered diabetic and used. Extracts were administered orally once a day for 7 days. Each animal was weighed once on day zero and the last day of the experiment to assess the effect of the extracts on body weight. On day 7, after obtaining their weights, the animals were sacrificed by over dose ether and the organs removed. The wet weight of the organs was taken and calculated as a percentage of their body weights. Tissue sections of each organ was fixed on slides (Obidoa et al., 2003) examined for histopathological changes.

Statistical analysis: Data was analysed using Student t-tests (SPSS Genstat Release 4.23DE PC). Difference between means was accepted significant at $P < 0.05$ using a 2-tailed distribution.

Results

Extraction: The extraction process yielded 9% w/w of the aqueous extract and 13% w/w of the ethanol extract.

Effect of extracts on visceral organs

Body and Organ weight: The extracts significantly ($P<0.05$) increased the body weights of treated animals after 7 days. The weight gain in rats treated with ethanol extract was greater than those treated with aqueous extract. The organ weights were not significantly increased (Table 1).

Table 1: Effect of extracts on body and organ weight

Treatment	Body weight		Gain in Weight	Organs (% of Body Weight)	
	Days 0	Days 7	(%)	Liver	Kidneys
EE	160	176.24	10.15 ± 0.35	3.05 ± 0.38	0.52 ± 0.03
WE	160	172.30	7.70 ± 0.21	2.71 ± 0.06	0.46 ± 0.43
Control	160	192.13	20.08 ± 0.49	3.16 ± 0.15	0.54 ± 0.02

n = 10, mean = ± S.E.M, EE = Ethanol Extract, WE = Water Extract

Histopathological changes: Histological changes observed in the liver showed interlobular branches of the hepatic artery (A) and hepatic portal vein (V). Sinusoid (S) separate cords of hepatocytes with vesicular nuclei (arrowhead). This normal histology was characterized by inconspicuous lobulation but with cords of hepatocytes, separated by sinusoids, making a centrifugal spread from the central blood vessels to the portal areas (Plate 1).

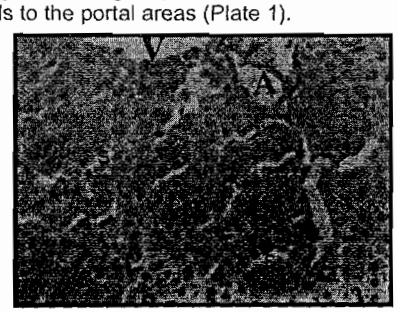

Plate 1: Liver section of rat injected with physiologic saline. H and E Stain, X 320

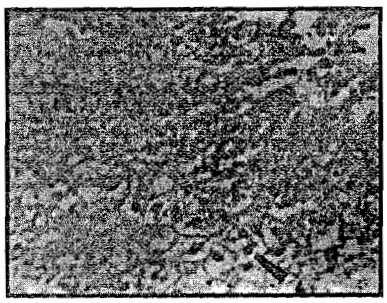

Plate 2: Liver section of diabetic, untreated rats (positive control). H and E Stain X 320

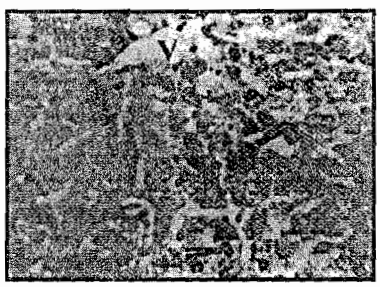

Plate 3: Liver section of diabetic rat treated with the water extract (WE750). H and E stain X 320

Liver section of diabetic, untreated rats showed severe disruption of the lobular architecture. The hepatocytes were moderately to severely vacuolated in the cytoplasm while the nuclei were condensed (pyknotic). The lesions were lobular, but more severe in the centrolobular area (Plate 2).

Liver section of diabetic rat treated with the water extract showed that many hepatocytes in the centrolobular areas had

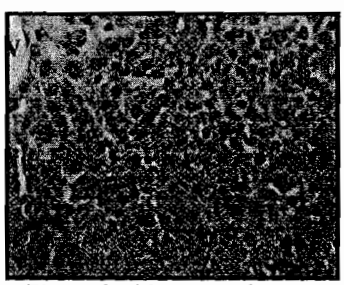

Plate 4: Liver section of diabetic rat treated with the alcoholic extract (EE750). H and E Stain, X 320

granular cytoplasm and vesicular nuclei. Only a few hepatocytes had pyknotic nuclei. This was a predominantly degenerative and mildly necrotic change (Plate 3).

Liver section of diabetic rats treated with the alcoholic extract showed mild distortion of the lobular architecture. The hepatocytes in the centrolobular areas had mildly granular cytoplasm

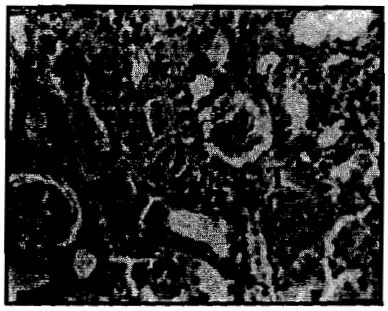

Plate 5: Kidney section of non-diabetic, untreated rat. H and E Stain, X 320

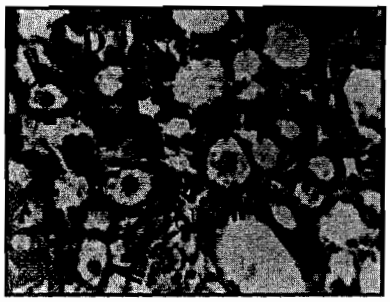

Plate 6: Kidney section of diabetic, untreated rat. H and E Stain, X 320

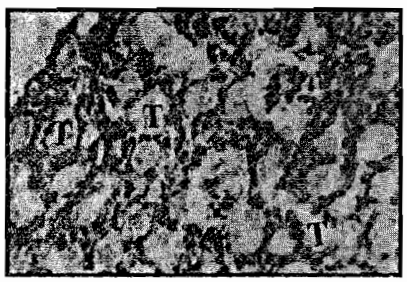

Plate 7: Kidney section of diabetic rat treated with water extract (WE750) H and E Stain, X 320

and vesicular nuclei. Only few, focally located hepatocytes had pyknotic nuclei (Plate 4).

Kidney section of rats that received normal saline showed normal histology with renal corpuscles (glomerulus and Bowman's capsule) focally distributed in the cortical areas and renal tubules in both the cortex and the medulla having normal epithelial cells (Plate 5).

Kidney section of the diabetic untreated rats showed moderate to severe degeneration and necrosis of the tubular epithelial cells. Many of the tubules especially around the medullary junction contained bright pink precipitates in their lumina (hyaline casts), which is indicative of severe epithelial injury (Plate 6).

Kidney section of diabetic rats treated with the water extract of the plant was moderately to severely hyperaemic, while the tubules were moderate to severe degeneration of the tubular epithelial cells (Plate 7). Necrosis of epithelial cells was very mild and there were no tubular casts.

Discussion

Treatment of rats with alloxan, which induced diabetes in this study, resulted in significant ($P < 0.05$) suppression of body weight gain relative to control rats, which were not treated with alloxan. This suppression in body weight was due to the wasting of muscle mass similar to wasting of muscle mass in human diabetic subjects. The main determinant of the creatinine pool is the muscle mass. Wasting diseases e.g. diabetes will influence the production and therefore the plasma concentration and excretion of creatinine (Goldman and Moss, 1959). More so, the suppression in body weight may be due to loss of appetite caused by the alloxan observed in the animals after injection of alloxan. Suppression in body weight gain in animals exposed to various organic solvents and compounds have been reported (Tatrai *et al.*, 1981; Ungavry *et al.*, 1981 and Uemura *et al.*, 1995).

None of the visceral organs showed any remarkable change in weight when compared with their controls. Crampton *et al.*, (1977) suggested that liver enlargement unaccompanied by sustained induction of drug metabolising enzyme activity might be an index of hepatotoxicity. But enlargement of the liver accompanied by induction of microsomal enzymes is an adaptive response and beneficial to the body. In this study, even though there was an increase in relative liver weight, it was not significant. The insignificant weight increase observed in this study may indeed be an adaptive response.

Inflammation and necrotic changes observed in the liver and kidney treatment with extracts may be due to free radical metabolites. Several authors (Slater, 1984; Halliwell and Gutteridge, 1986; Borg, 1993 and Comporti, 1993) have implicated free radicals in tissue injury.

The result of the liver and kidney histopathologic tests showed that the extracts caused some circulating disturbances, mild degenerative/necrotic, inflammatory and degenerative changes which could be caused by contaminants or the antihypoglycaemic active principle contained in the extract.

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