

Ameliorative Effects of *Cussonia arborea* Methanol Root Bark Extract on Histomorphology of Pancreas of Alloxan-Induced Diabetic Rats

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Abstract

Diabetes mellitus is an endocrine and a metabolic disease resulting from the destruction of pancreatic beta cells; thus assessment of the pancreas in diabetic rats is important in monitoring therapy. The hereby study assessed pancreatic status of diabetic rats treated with methanol root bark extract of *Cussonia arborea*. A total of seventy two (72) male albino wistar rats weighting between 100-105 g were assigned into six (6) groups of twelve (12) rats per group. Groups 1-5 were diabetic infected by single intraperitoneal injection with alloxan monohydrate, at the dose of 160 mg/kg and treated with 62.5, 125, 250 mg/kg bw of the extract, 2 mg/kg bw glibenclamide and 10 ml/kg distilled water (DW) respectively, while the non diabetic rats, represented by Group 6, received 10 ml/kg DW and served as normal control rats. The treatment was applied daily through the oral route for 84 days. At the end of the experiment, the pancreas organs were acquired under light ether anaesthesia for histomorphometric assessment. The results indicated that the cells of the islet of langerhans of the diabetic untreated rats (Group 5) were severely depleted when compared to that of the normal rats (Group 6). The islet cells of the diabetic rats treated with 125 mg/kg *Cussonia arborea* extract (Group 2) was comparable to that of the diabetic rats treated with glibenclamide (Group 4) and the normal control rats. It was concluded that the methanol extract of *C. arborea*, especially at the dose of 125 mg/kg, ameliorated pancreatic lesions induced by diabetes occasioned by alloxan.

Keywords: *Cussonia arborea*, diabetic rat, histopathology, pancreas

Introduction

Diabetes mellitus is an endocrine and a metabolic disease consequent upon insulin lack and sequel to hyperglycemia (Celik *et al.*, 2002). Hyperglycemia itself produces the classical signs and symptoms of polyuria, polydipsia, polyphagia and glucose urea. Prolonged hyperglycemia predisposes diabetics to oxidative stress, which precipitates complications such as nephropathy, neuropathy, angiopathy and retinopathy (Dewanjee *et al.*, 2008). The disease is very common in humans and animals with prevalence rate varying from 1-50% (King and Rewers, 1993).

Experimental diabetes in rodents can be induced by alloxan monohydrate which causes necrosis of the pancreatic beta cell. This will result to elevation of fasting blood glucose levels since insulin production is impaired (Etuk, 2010). Insulin is responsible for metabolizing "dragging" blood glucose to the cells. Inactivation of glucokinase, production of free radicals and changes in the normal physiology of intracellular calcium have been proposed as the possible cause of the toxic action of alloxan on pancreatic beta cells (Szukudelski, 2001). The pancreatic beta cells readily uptake the alloxan (because of its structural resemblance to glucose) and thus precipitates the toxic injury to the cells (Lenzen, 2008).

For a very long time now, plants have been used in the management of diabetes mellitus. However, these effects are usually exaggerated, unscientific or unstandardized. *Cussonia*

arborea is a tropical plant belonging to the family of Araliaceae, with about twenty species (Tennant, 2010). Folklorically, the plant is used in the treatment of malaria, fungi diseases, chronic diarrhea and diabetes (Kinsangau *et al.*, 2007; Amadou *et al.*, 2008; De Villiers *et al.*, 2010). The antioxidant potentials of the plant had earlier been reported (Aba *et al.*, 2014).

The current study was therefore tailored to investigate the possible ameliorative effects of treating alloxan-induced diabetic rats with different graded doses of methanol root bark extract of *Cussonia arborea* on histomorphometry of the pancreas.

Materials and Methods

Experimental animals

Male albino rats' weighting between 100-105 g were obtained from the Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka laboratory animal house. The rats were randomly assigned into 6 groups of 12 rats per group. Diabetes was induced in groups 1-5, while the rats in group 6 served as normal (non infected) control rats. The rats were acclimatized for two weeks. The environmental temperature where the animals were housed varied between 28-32 °C. The animals were kept in stainless wire mesh cages and provided with good clean water *ad libitum*. They were fed with standard commercial feed (Guinea^R growers). The experimental

protocol used in this study was approved by the ethics committee of the University of Nigeria, Nsukka, and conforms with the guide to the care and use of animals in research and teaching of University of Nigeria, Enugu state, Nigeria (ECUN: 2015/58/702).

Plant material

The root bark of the plant material (*Cussonia arborea*) used in this study was collected from Orukpa Local Government Area of Benue state and identified by a plant taxonomist, at the International Centre for Ethnomedicine and Drug Development, Echara, Aku Road, Nsukka.

Preparation of the plant extract

Cold maceration method of extraction was employed. The root bark of *C. arborea* was air dried at a very low intensity of sunlight to avoid denaturation of the active ingredients. It was pulverized and stored in an air tight container pending its usage. About 1 kg of the powdered stem bark was soaked in 5 l of 80% methanol (Sigma Aldrich, UK) with intermittent shaking every 2 h for 48 h. The mixture was filtered using Whatmann No 1 filter paper. The filtrate was concentrated using rotary evaporator and the extract stored as *C. arborea* extract (CAE) at 4°C.

Induction of experimental diabetes mellitus

Diabetes was induced in rats using the method described by Prince and Menon (1998). The rats were injected with alloxan monohydrate (Sigma Aldrich, UK) dissolved in distilled water at the dose of 160 mg/kg body weight, intraperitoneally, after overnight fasting (18 h). Before the injection with alloxan monohydrate, the FBS levels of the rats were taken using Accu-Check glucometer. This was done by tail snip of the rats and allowing blood to drop on the glucometer strip. The value was read off on the screen of the glucometer. After induction, the rats were kept in clean stainless cages and fed with commercial feed and were also given clean water for about 2 days before they came down with diabetes. On the 2nd day, diabetes was confirmed. The rats were fasted overnight before the assessment of their glucose blood status on the 2nd day. The fasting blood glucose values above 7 mMol/L (126 mg/dl) were considered diabetic (Prince and Menon, 1998).

Treatments

The rats were treated as follows:

Group 1: Diabetic rats treated with 62.5 mg/kg *C. arborea* extract (CAE);

Group 2: Diabetic rats treated with 125 mg/kg CAE;

Group 3: Diabetic rats treated with 250 mg/kg CAE;

Group 4: Diabetic rats treated with 2 mg/kg glibenclamide;

Group 5: Diabetic rats treated with 10 ml/kg distilled water;

Group 6: Non diabetic rats treated with 10 ml/kg distilled water.

The rats were treated orally daily for eighty four (84) days. At the end of the experiment, the pancreases were collected under light ether (Sigma Aldrich, UK) anaesthesia into a bottle containing formol saline for histopathology processing

Histopathology examination

The histological examination of the tissues of the pancreas of male albino rats was done using the method of Drury *et al.* (1967).

Results and Discussion

Pancreas of normal control rats (Group 6) showed normal cells (arrow) (Fig. 1) of the islet of langerhans (H&E X400). The islet cells appeared fully populated (Fig. 1). The pancreas of diabetic untreated rats (Group 5) showed depopulation of the islet cells (arrow) (H&E X400). The cells of the islet of langerhans were severely depleted (Fig. 2). Pancreas of Group 1 rats (diabetic + 62.5 mg/kg extract) showed moderate population of the islet cells of the langerhans (arrow) (H&E X400).

The islet cells appeared moderately populated (Fig. 3). Histomorphometric assessment of pancreas of group 2 of rats (diabetic +125 mg/kg extract) showed good population of the islet cells of the langerhans (arrow)(H and E X400) comparable to the normal control rats (Fig. 4). Pancreas of Group 3 rats (diabetic + 250 mg/kg extract) showed cytoplasmic vacuolations (arrow) and moderate population of the islet cells of the langerhans (H&E X400) (Fig. 5), while the pancreas of Group 4 rats (diabetic + 2 mg/kg glibenclamide) showed good population of the islet cells of the langerhans (arrows) (H&E X400) (Fig. 6).

Significant loss of the cells of the islets of langerhans of the pancreas of diabetic untreated rats (Fig. 2) was attributed to the single intraperitoneal injection of alloxan monohydrate at the dose of 160 mg/kg body weight. The formation of superoxide radicals by alloxan is responsible for destruction of pancreatic beta cell (Szukuldeski, 2001). These free radicals dismutate to hydrogen peroxide with a simultaneous flooding of cytosolic calcium ions, which cause speedy destruction of pancreatic beta cells of the islets of langerhans. This is sequel to a significant decrease in endogenous insulin secretion and the resultant increase in the blood glucose levels (Akuodor *et al.*, 2014).

Observation of less severe degeneration and necrosis of pancreatic islet cells of the langerhans in the diabetic rats treated with *C. arborea* was attributed to the effect of the extract. These probably indicate that the extract may have prevented the free radicals generated by the alloxan

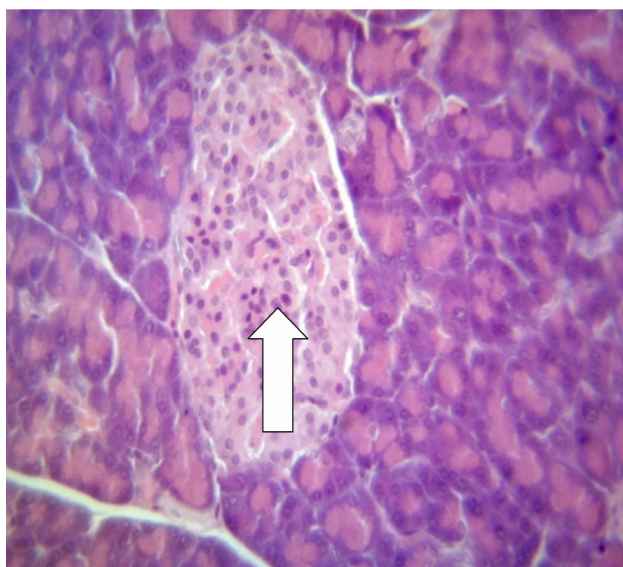


Fig. 1. Pancreas of normal control rats (Group 6)



Fig. 2. Pancreas of diabetic untreated rats (Group 5)

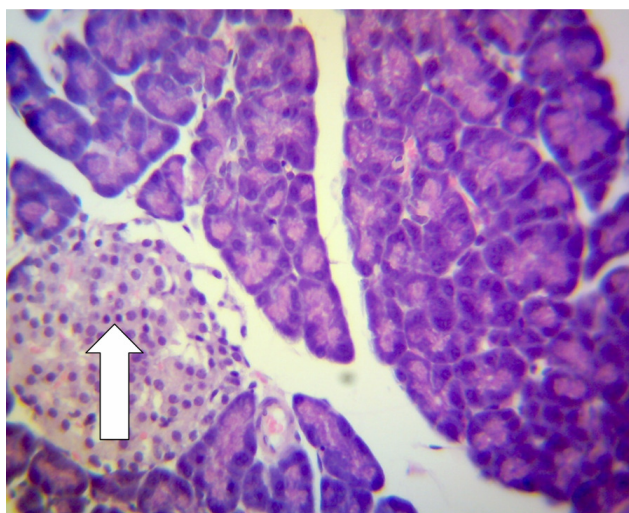


Fig. 4. Pancreas of Group 2 rats (diabetic + 125 mg/kg extract)

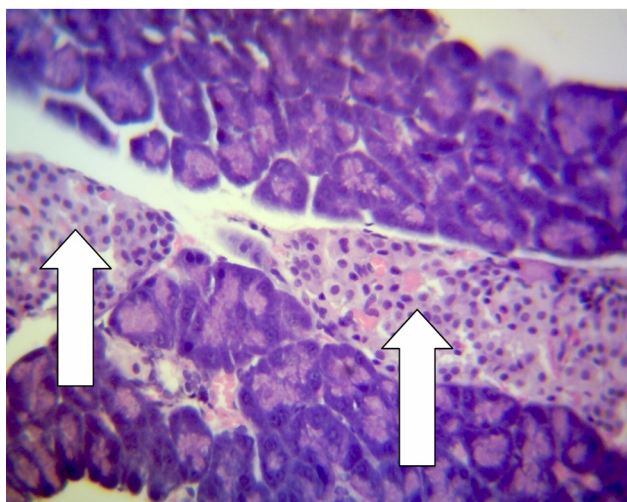


Fig. 6. Pancreas of Group 4 rats (diabetic + 2 mg/kg glibenclamide)

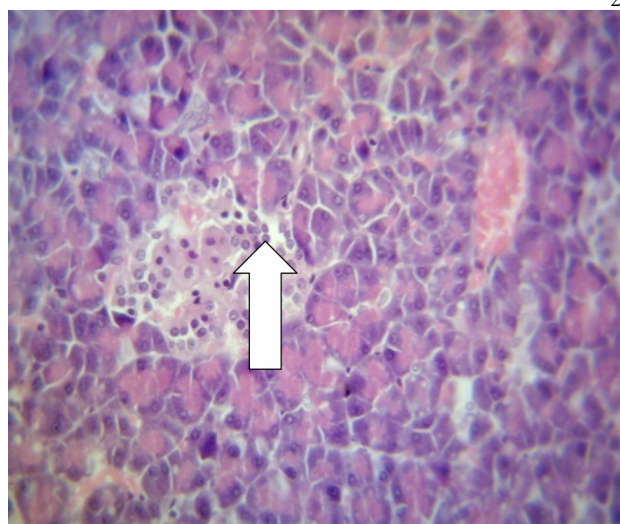


Fig. 3. Pancreas of Group 1 rats (diabetic + 62.5 mg/kg extract)

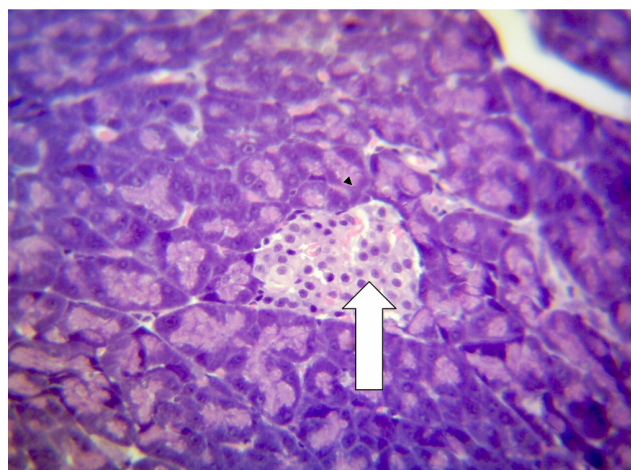


Fig. 5. Pancreas of Group 3 rats (diabetic + 250 mg/kg extract)

monohydrate in destroying the islet cells. Antioxidant potentials of *C. arborea* had earlier been reported (Aba *et al.*, 2014).

Antioxidants are known to mop up free radicals (Aba *et al.*, 2015). It is also possible that the extract may have stimulated the regeneration of the islet cells. Fernandez-Alvarez *et al.* (2004) reported a regeneration of beta cell mass following treatment of streptozotocin-induced diabetic rats with tungstate for one month. The pancreatic islet cells of the diabetic rats treated with 2 mg/kg glibenclamide showed good population, indicating that the alloxan-induced lesion in this group of rats was mild. The destructive effects of alloxan on the pancreatic cells have been widely reported (Szukudelski, 2001; Lenzen, 2008; Akuodor *et al.*, 2014) but still need to be better understood.

Conclusions

Based on the hereby results, it was concluded that treatment of diabetic rats with methanol root bark extract of *C. arborea* (especially at the dose of 125 mg/kg) for 84 days mitigated injury occasioned by alloxan to the pancreas.

Acknowledgements

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