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Adansonia Digitata (Baobab) Amelioratethyroid Gland Disorder of Alloxan-Induced Diabetes Model.

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ABSTRACT

Diabetes and thyroid disorders have been shown to mutually influence each other because of the role of thyroid gland in the regulation of body metabolism. This work is therefore designed to study the effect of aqueous leaf extract of *Adansonia digitata* (baobab) on the thyroid gland of alloxan-induced diabetes. Twenty-eight Wistar rats, weighing 120g to 195g were divided into seven groups (n=4). Sham control, Diabetes control, Diabetes + Low dose, Diabetes + High dose, Diabetes + metformin, Low dose, and High dose. Diabetes was induced with a single dose of 150mg/kg alloxan monohydrate and animals were treated with *Adansonia digitata* for 14 days. This study indicated that the leaf extract of *Adansonia digitata* increase serum triiodothyronine levels and reversed the histological damage to the thyroid gland. Therefore, *Adansonia digitata* could be used to manage metabolic dysfunctions in the diabetic thyroid gland.

Keywords: Diabetics, Thyroxine, *Adasonia Digitata*, Metformin, Triiodothyronine

INTRODUCTION

Diabetes mellitus and thyroid diseases are two common endocrine disorders that affect multiple organs in the body and mutually affect each other (Hage *et al*. 2011; Wang et al. 2018). Thyroid hormones regulates carbohydrate metabolism and pancreatic function while diabetes mellitus affect TSH production, impairs the responds of TSH to TRH, reduced T3 level, and impairs the conversion of T4 to T3 (Hage *et al*. 2011). On the other hand, Hyperthyroidism increases glucose production, absorption, and utilization thereby leading to hyperinsulinemia, abnormal glucose tolerance, and peripheral insulin resistance (Lambadiari *et al*. 2011).

In developing countries, natural products especially from plants origin have been of great sources of relief for disease. One of such plant is *Adansonia digitata* with "Baobab" as English name. It belongs to the Malvaceae family. The different parts of the *A.digitata* provide food, shelter, clothing, medicine, and sources of raw materials for industrial use (Venter and Venter, 1996; Yakubu et al. 2020). The antioxidant and anti-inflammatory properties of the tree makes it relevant in the treatment of different types of ailments (De-Caluwe *et al.* 2010; Kamatou *et al.* 2011). Recent phytochemical analysis of its leaves revealed that they contain a rich repertoire of reducing sugars, flavonoids, terpenoids, saponins, tannins, alkaloids, anthraquinones, steroids, resins, phenols, and cardiac-active glycosides (Abiona et al. 2015; Finbarrs-Bello et al. 2020). Traditionally in some parts of Africa, the leave is used to treat fatigue, dysentery, diseases of the urinary tract, opthalmia and otitis (Abiona et al. 2015). Moreover, it is used as a tonic, and for insect bites Guinea worm and internal pains, (Sidibe and Williams, 2002). Baobab leaves are potential source of protein used to complement amino acid to improve the overall protein quality in local diet (Nordeide et al. 1996). The

leaves are sources of minerals, iron and calcium (Barminas et al. 1998; Boukari et al. 2001). This study was aimed at determining the effects of aqueous extract of *Adansonia digitata* on the thyroid gland in alloxan-induced diabetic Wistar rats.

MATERIAL AND METHODS

Preparation of plant materials

Fresh leaves of *Adasonia. digitata* were procured from a local dealer in Kaduna State. It was identified by a botanist in the Department of Plant Sciences and Biotechnology, University of Nigeria, Nsukka. The extraction method described by Bello and Nwoso (2015) was used. 500g of the powdered plant material was soaked in 1500ml of distilled water for 72 hours. Separation of the plant extract was carried out using Whatman NO. 4 filter paper. The resulting extract was then concentrated using rotatory evaporator, and stored at 4°C before use.

Experimental Animals

A total of 28 adult male Wistar rats were purchased from the animal house of the University of Nigeria, Enugu Campus. The animals were bred in the animal facility of Enugu State University of Science and Technology College of Medicine, Enugu. The animals were housed in netted iron cages and were provided easy access to food (Grower's mesh, New market Enugu) and water ad libitum; and were maintained under standard laboratory conditions (temperature 20°C to 24°C, with relative humidity of 60-70% under 12 hours light and day cycles) and allowed to acclimatize for two weeks prior to the experiment. At the end of acclimatization, the animals were randomly divided into seven (7) groups of four (4) animals each. They were labeled as:

Sham control - 0.1ml/kg/bw normal saline/14days
Diabetes control - Alloxan (150mg/kg)/2days
Diabetes + Low dose -150mg/kg/bw Alloxan +400mg/kg/bw *Adansonia digitata* extract
Diabetes + High dose -150mg/kg/bw Alloxan +600mg/kg/bw *Adansonia digitata* extract
Diabetes + Metformin -150mg/kg/bw Alloxan + 150mg/kg/bw Metformin
Low dose - 400mg/kg/bw *Adansonia digitata* extract only
High dose- 600mg/kg/bw *Adansonia digitata* extract only

The body weights of the animals were obtained before and after acclimatization, and at weekly intervals during the experiment. The protocol for conducting the in-vivo study in Wistar rats was approved by the Institutional Animal Ethical Committee (IAEC), Enugu State University of Science and Technology.

Induction of Diabetes using alloxan

Stock solution of alloxan was prepared by dissolving alloxan monohydrate (Sigma-Aldrich, USA) (0.9g) in 6ml of distilled water, and diabetes was induced by single intra-peritoneal injection of alloxan monohydrate (150mg/kg). The volume of the solution containing 150mg/kg given to each rat was determined by its weight. After a period of two days, the rats with blood glucose level greater than 200mg/dl was considered diabetic and used for the research work. The method of Mohammad and Hauwa'u (2013) was adopted in the study with slight modification.

Hormonal assay

At the end of the experiment, blood samples were collected via cardiac puncture from all rats. Serum was then separated and stored at -20°C until the hormonal assay. Serum levels of thyroid hormones thyroxine and triiodothyronine concentrations were determined by ELISA (Enzyme-linked Immune-sorbent Assay) by Micallef *et al.* (1995)

Histopathological study

Twenty four hours after the last treatment, the animals were sacrificed under anasthesia (ketamine hydrochloride). Sections of the thyroid were collected for histopathological examination. The samples were fixed in 10% phosphate buffered formalin for a minimum of 48 hours. The tissues were subsequently trimmed, dehydrated in 4 grades of alcohol

(70%, 80%, 90% and absolute alcohol), cleared in 3 grades of xylene and embedded in molten wax. On solidifying, the blocks were sectioned, 5µm thick with a rotary microtome, floated in water bath and incubated at 60℃ for 30 minutes. The 5µm thick sectioned tissues were subsequently cleared in 3 grades of xylene and rehydrated in 3 grades of alcohol (90%, 80% and 70%). The sections were then stained with Hematoxylin for 15 minutes. Blueing was done with ammonium chloride. Differentiation was done with 1% acid alcohol before counterstaining with Eosin. Permanent mounts were made on degreased glass slides using a mountant; DPX.The prepared slides were examined with a Motic™ compound light microscope (Motic BA410E Elite Research Compound Microscope; Motic Asia, Hong Kong) using x4, x10 and x40 objective lenses. The photomicrographs were taken using a Motic™ 5.0 megapixels microscope camera at x160 and x400 magnification

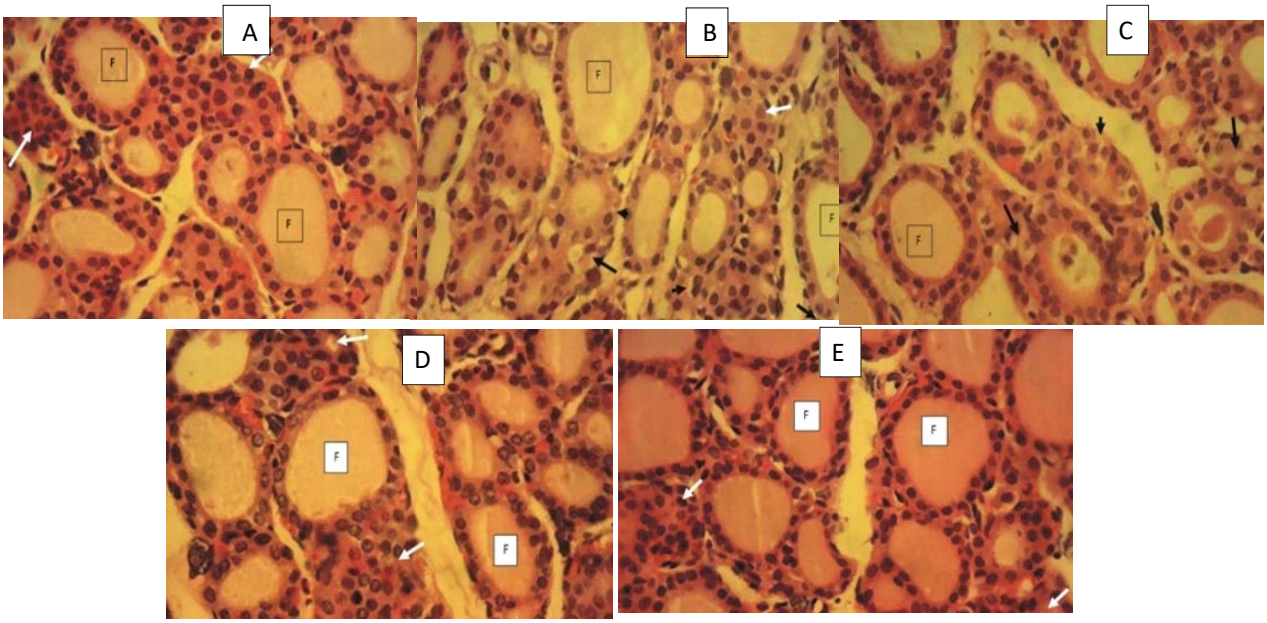
Statistical Analysis

Data obtained was expressed as the mean standard deviation. They were analyzed using Statistical Package for Social Sciences (SPSS version 21; IBM SPSS, Chicago, Illinois, USA) software package. Values were analyzed as Mean± SD using one way ANOVA with Tukey post hoc test. The level of significance was set to $p<0.05$.

RESULTS AND DISCUSSION

***Table 1: Adansonia digitata* increased triodothyronine concentration**

GROUPS	TRIODOTHYRONINE (T3)	THYROXINE (T4)
Sham control	1.02±0.16	4.43±2.03
Diabetic control	1.02±0.16	2.42±1.32
Diabetic + Low dose	1.07±0.16	2.57±1.04
Diabetic + High dose	1.09±0.17	1.99±1.56
Diabetic + Metformin	0.57±0.22	0.87±0.27*
Low dose extract only	1.44±0.45*	2.52±1.12
High dose extract on	1.47±0.43*	2.60±1.11



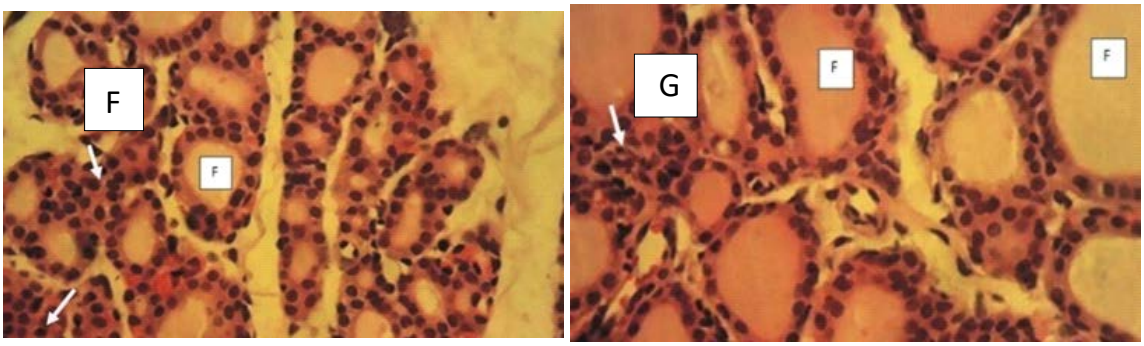


Figure 1: Section of thyroid gland
(A) Sham control - 0.1ml/kg normal saline/14days **(B)** Diabetes control (Alloxan, 150mg/kg).**(C)** Diabetes + Low dose -150mg/kg/bw Alloxan +400mg/kg/bw *Adansonia digitata* extract **(D)** Diabetes + High dose -150mg/kg/bw Alloxan +600mg/kg/bw *Adansonia digitata* extractg/bw **(E)** Diabetes + Metformin -150mg/kg/bw Alloxan + 150mg/kg/bw Metformin **(F)** Low dose - 400mg/kg/bw *Adansonia digitata* extract only. **(G)** High dose- 600mg/kg/bw *Adansonia digitata* extract only.

In this study, we used the leaf extract of *Adansonia digitata* on alloxan-induced diabetic rats to investigate the relationship between *A. digitata* leaves extract and thyroid gland of diabetes mellitus Wistar rats with an aim to establish its usefulness in the treatment of diabetes with co-morbid thyroid dysfunction. The thyroid gland of sham control, low- and high- dose animals showed normal histoarchitecture with parafollicular cells (arrow) and thyroid follicular cells observed. The parafollicular cells are mostly located within the basal aspects of the follicular cells and the basement membrane of the follicles. The follicles (F) are line by cuboidal to columnar lining cells with their polarity directed towards the lumen of the follicles. The thyroid gland of the diabetic control animals showed vacuolar degeneration and random individual necrosis of the thyroid follicular cells and the parafollicullar cells. The affected cells appear swollen with numerous minute clear intracytoplasmic vacuoles while the *A. digitata* and metformin treated groups recuperated and normal histo-morphology of thyroid gland cells were observed. Follicles (F); Cells (White arrow) as seen in fig.1.

Sathish and Mohan (2003) revealed that diabetes may induce a "low T3 state". In contrast to this finding, differences were not found in the serum levels of triodothyronine (T3) of both sham control and diabetic control groups (Table 1). However, animals that received *A. digitata* had increase T3 even though the increase was not significant when compared to the sham control and diabetic control. Significant differences were observed in groups that receive *A. digitata* without diabetes and diabetic + metformin group. This implies that *A. digitata* had potency to increase serum triodothyronine (T3) levels and also had the capacity to restore the hormone back to its normal levels in diabetic conditions.

More proof of this fact could be seen in the histology of the thyroid gland where the medium and high dose of the aqueous leaf extract of *Adansonia digitata* were sufficient to reverse the thyroid toxicity brought about by Alloxan administration.Although diabetes and *A. digitata* led to reduction of thyroxine (T4) level, significant differences were not found in serum levels of T4 in all groups except in diabetic + metformin group when compared with the sham control suggesting that metformin has the ability to reduce serum T4 level. The reduction in T4 level of animals treated with metformin contrast previous works by Hu *et al.* (2017) where metformin led to significant increase in T4 level of animals and Anil *et al.*(2016) where changes was not observed among metformin treated and non-treated animals.

CONCLUSION

The present study indicates that treatment of alloxan-induced diabetes rats with *Adansonia digitata* extracts increased thyroid hormones concentrations, and restored the diabetic thyroid gland to its normal state.

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