

## Antihyperglycaemic and Antihyperproteinaemic Activity of Extracts of *Picralima nitida* Seed and *Tapinanthus bangwensis* Leaf on Alloxan-Induced Diabetic Rabbits

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**ABSTRACT:** Coconut water extract of *Picralima nitida* seed and aqueous extract of *Tapinanthus bangwensis* leaf were investigated for their antidiabetic activities on some biochemical parameters (glucose, protein) associated with diabetes in both the serum and tissues of experimental animals using alloxan-induced diabetic rabbits as model. The rabbits were fasted overnight before they were given a single intraperitoneal injection of aqueous alloxan monohydrate (Sigma, USA) at a dose of 300 mg/kg body weight to make them diabetic. The experimental rabbits (chinchilla) were grouped into six and extracts administered orally, once daily for five weeks. Groups 1 and 2 (non-diabetic) received only distilled water and coconut water respectively, group 3 (diabetic) received 200 mg/kg body weight aqueous extract of *T. bangwensis* leaf, group 4 received 400 mg/kg body weight of coconut water extract of *P. nitida* seed, groups 5 and 6 (diabetic) received only distilled water and coconut water respectively. The results revealed that the extracts independently lowered significantly ( $p < 0.05$ ) the blood glucose and protein levels of the diabetic rabbits. Both extracts significantly ( $p < 0.05$ ) increased the tissue protein. Overall, aqueous extract of *T. bangwensis* leaf and coconut water extract of *P. nitida* seed independently possesses insulin-like properties as demonstrated by their antidiabetic actions, hence, may be good herbal extracts in the management of diabetes.

**KEYWORDS:** Alloxan, Antihyperglycaemic, Antihyperproteinaemic, Diabetes, *Picralima nitida*, *Tapinanthus bangwensis*.

### 1 INTRODUCTION

Diabetes mellitus referred to simply as diabetes, is a syndrome characterized by disordered metabolism manifesting abnormally high blood sugar (hyperglycaemia) resulting from insufficient levels of insulin, the principal hormone that regulates uptake of glucose from the blood into most cells primarily muscle and fat cells [1].

Diabetes is one of the world's commonest diseases today with about 171 million people being affected by the disease [2]. The figure is ever increasing and it has been predicted that by 2030, over 366 million people would be living with diabetes if current trend continues [3].

The orthodox approach of managing diabetes is faced with a lot of difficulties, this is partly because several of the drugs, aimed in managing diabetes pose a significant risk of inflicting heart disease and no pill or injection to date is able to address the problem of dying pancreatic beta cells, a fundamental dysfunction in diabetes.

The management of diabetes concentrates on keeping blood sugar levels as close to normal as possible, without causing hypoglycaemia.

Consequent upon the problems associated with orthodox approach in the management of diabetes, effort is now geared towards the traditional approach of curing/managing diabetes. It is assumed that this approach is safer and more natural.

The use of herbal medicines for the treatment of diabetes mellitus has gained importance throughout the world. The World Health Organization also recommended and encouraged this practice especially in countries where access to the conventional treatment of diabetes is not adequate [4].

In Nigeria, many herbalists have claimed to use the coconut water extract of *Picalima nitida* (Akuamma) seed and aqueous extract of *Tapinanthus bangwensis* (Mistletoe) leaf for the treatment for various diseases, including diabetes. In Nigeria, several herbal preparations from leaves and twigs of mistle-toes e.g. *T. bangwensis* (Engl. and K. Krause) Danser have become popular for the treatment of variety of diseases, such as diabetes and hypertension, which have been reported to be on the increase in the country [5]. However, information is scanty in open scientific literature to support the folkloric use of these plants as antidiabetic agents.

## **2 MATERIALS**

### **2.1 COLLECTION AND PROCESSING OF PLANT MATERIALS**

The seeds of *Picalima nitida* (Stapf) T. Durand & H. Durand were purchased from herbseller at 'Baboko market', Ilorin, Nigeria while the leaves of *Tapinanthus bangwensis* (Engler & K. Krause) Danser from citrus tree (host plant) were obtained from a farm settlement in 'Ila-Orangun', Osun State, Nigeria. The plants were authenticated at Forest Research Institute of Nigeria (FRIN) Ibadan, Nigeria. Voucher samples were preserved in the Institute's Herbarium ( FHI 109955 and FHI 109972 for *P. nitida* and *T. bangwensis* respectively). The seed coats of the *P. nitida* seeds were removed with the hand and the *T. bangwensis* leaves were washed free of sand and debris. The seeds and leaves of the *P. nitida* and *T. bangwensis* respectively were air- dried for two weeks at room temperature (  $28^{\circ}\text{C} \pm 0.02$ ) and pulverized using an electric blender ( Holt Star, Model BE 768-2, John Holt product, UK).

### **2.2 ANIMALS AND FEED**

The experimental rabbits (36), (chincilla breed) of mixed sexes from Goshen rabbit farm, Osogbo, Nigeria were randomized into six groups of six animals each were used in this study. Rabbit pellets (a product of Guinea Feeds, Ibadan, Nigeria) and water were available to the animals *ad libitum* throughout the experimental period. The experimental rabbits were acclimatized for a week. Experimental animals were handled according to the stated guidelines of Ethical Committee on the ethical use of animals in research.

## **3 METHODS**

### **3.1 PREPARATION OF PLANTS EXTRACTS**

Solvent extract of pulverized seeds of *P. nitida* and leaves of *T. bangwensis* were prepared by separately suspending 500g each of the powdered samples in 1000 ml of coconut water and distilled water respectively using the modified method of Gray and Flatt [6]. The mixtures were left to infuse overnight and were thereafter vigorously shaken for 3 hours using a wrist hand shaker for thorough extraction. The extracts were filtered with Whatman No. 1 filter paper and the filtrates concentrated in a rotary evaporator to obtain semi solid extracts. Calculated amount of the extract was weighed and thereafter used to determine the concentrations of the extracts administered to the different groups of the experimental animals using the appropriate solvent.

### **3.2 INDUCTION OF DIABETES**

The rabbits were fasted overnight before they were given a single intraperitoneal injection of aqueous alloxan monohydrate (Sigma-Aldrich, USA) at a dose of 300 mg/kg b. w. to make them diabetic. After 6 hours, they were given 5% D - Glucose solution to drink to counter the hypoglycaemic shock phase [7]. The fasting blood glucose level of blood samples drawn from the tail vein puncture was determined after 72 hours using Onetouch ultraeasy glucometer (Lifescan Johnson and Johnson Company, Milipitas). Rabbits showing blood glucose level greater than 180 mg/dL were selected for the study [8] to indicate diabetic rabbits.

### 3.3 EXPERIMENTAL DESIGN AND EXTRACT ADMINISTRATION

The experimental rabbits (mix sexes) were grouped into six (6). Extracts were administered orally at the dose of 200 mg/kg and 400 mg/kg for *Tapinanthus bangwensis* and *Picralima nitida* respectively, once daily for five (5) weeks. The dosages were arrived at with series of preliminary studies to establish the concentration at which the extracts exhibited the safest and highest hypoglycaemic activities. The animals were grouped and treated as follows:

- A: Non diabetic rabbits placed on distilled water only (NDDW)
- B: Non diabetic rabbits placed on coconut water only (NDCW)
- C: Diabetic rabbits placed on distilled water extract of *T. bangwensis* leaf (DDWT)
- D: Diabetic rabbits placed on coconut water extract of *P. nitida* seed (DCWP)
- E: Diabetic rabbits placed on distilled water only (DDW)
- F: Diabetic rabbits placed on coconut water only (DCW)

### 3.4 BLOOD SAMPLES COLLECTION AND PREPARATION

At the end of the fifth week of experimentation, the rabbits were anaesthetized with chloroform. The jugular vein was exposed and with a sterile knife, the rabbits were bled and whole blood sample was collected from each group of the animals into sterile plain bottles and allowed to clot for one hour and were thereafter centrifuged at 2000 rpm for 10 minutes [9] to obtain the serum which was then collected in universal sample bottles and stored in the freezer until required.

### 3.5 COLLECTION AND WEIGHING OF ORGANS

The animals were dissected after the collection of the blood samples and each of their kidneys, hearts and livers were removed. The organs were blotted in clean tissue paper, weighed, and thereafter kept frozen until required for homogenization.

### 3.6 HOMOGENIZATION OF THE ORGANS OF THE EXPERIMENTAL ANIMALS

A known weight of each of the organ (liver, kidney and heart) of the experimental animals was put into the mortar and homogenized with pestle in an aliquot of ice – cold 0.25 M sucrose solution until a smooth homogenate was obtained.

### 3.7 DETERMINATION OF BIOCHEMICAL PARAMETERS

Blood glucose concentrations were read using Onetouch ultraeasy glucometer (Lifescan Johnson and Johnson Company, Milipitas, CA) following the procedures as outlined by the manufacturer. Protein concentrations in the serum and tissue homogenates were determined as described by Gornal *et al*, [10].

### 3.8 STATISTICAL ANALYSIS

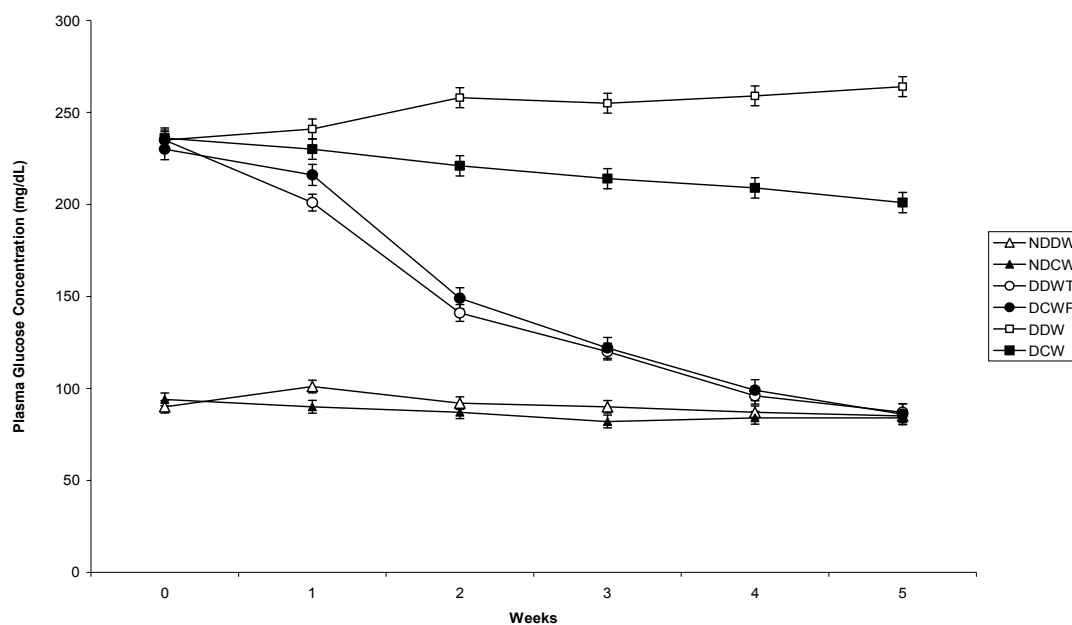
All data were subjected to analysis of variance using the model for randomized complete block design [11]. Significant differences between treatment means were determined at 5% confidence level using Duncan's Multiple Range Test (SPSS 16).

## 4 RESULTS

The blood glucose levels in untreated diabetic rabbits (DDW, DCW) when compared with normal rabbits were significantly ( $p < 0.05$ ) raised (Figure 1). Administration of aqueous extract of *T. bangwensis* leaf and coconut water extract of *P. nitida* seed respectively decreased blood glucose level in diabetic rabbits (DDWV, DCWP).

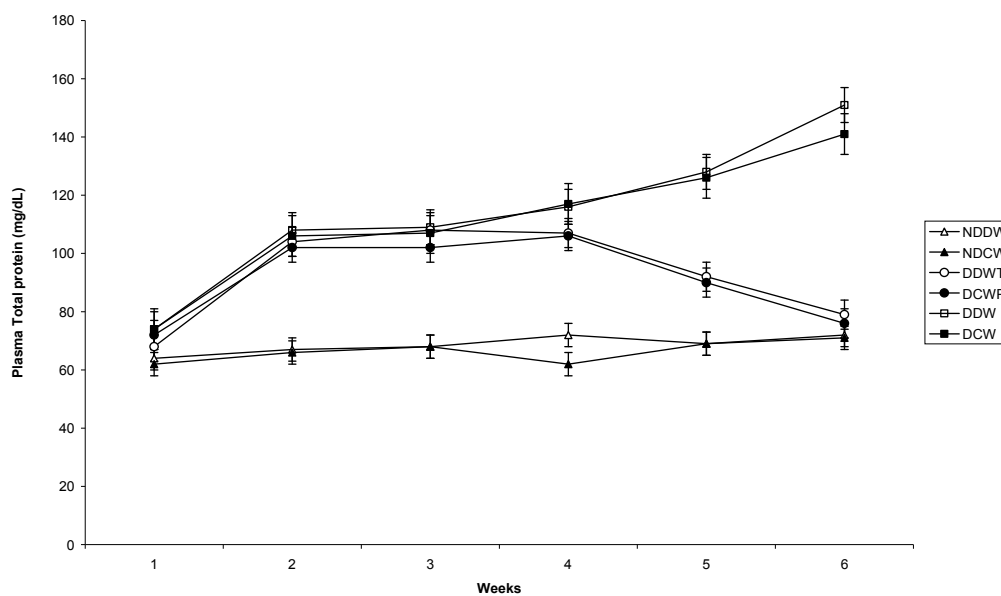
Except for the extract treated rabbit groups (DDWT and DCWP), the untreated rabbit groups exhibited a significant increase ( $p < 0.05$ ) in the plasma protein concentration in comparison to those of the control (Figure 2).

There was also significant ( $p < 0.05$ ) reduction in the protein concentration in the organs of the diabetic untreated rabbits (DDW, DCW) (Figure 3) in comparison to those of the extract treated groups (DDWT and DCWP) though they also exhibited reduction in the protein concentration when compared with the control (NDDW and NDCW).



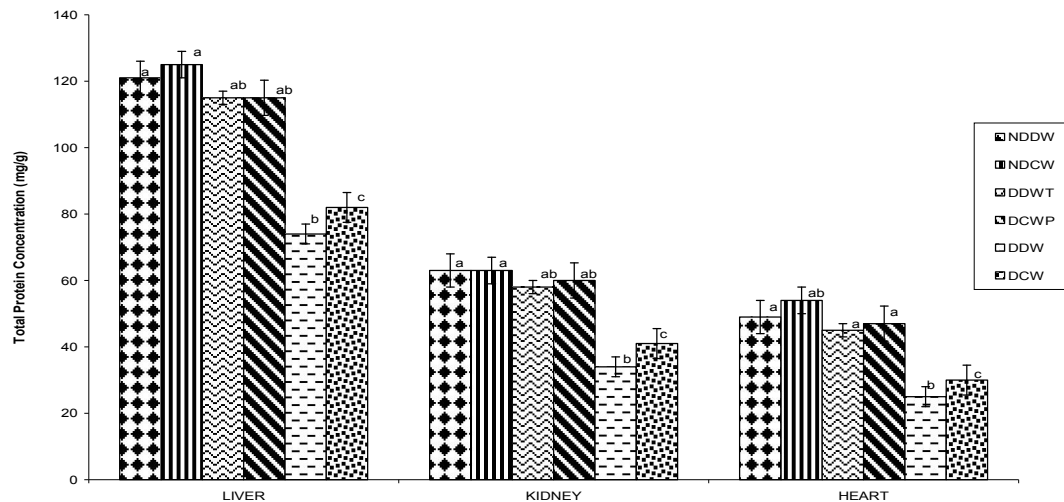
**Fig. 1. Plasma Glucose (mg/dL) Concentration of Alloxan - Induced Diabetic Rabbits Placed on Aqueous Extract of *T. bangwensis* Leaf and Coconut Water Extract of *P. nitida* seed over a Period of Five Weeks**

Values are Means of Six Determinations  $\pm$  S. E. M.



**Fig. 2. Plasma Total Protein Concentration (mg/dL) of Alloxan-Induced Diabetic Rabbits Placed on Aqueous Extract of *T. bangwensis* Leaf and Coconut Water Extract of *P. nitida* seed over a Period of Five Weeks**

Values are Means of Six Determinations  $\pm$  S. E. M.



**Fig. 3.** Total Protein Concentration (mg/dL) of Alloxan –Induced Diabetic Rabbits Placed on Aqueous Extract of *T. bangwensis* Leaf and Coconut Water Extract of *P. nitida* seed over a Period of Five Weeks. Values are Means of Six Determinations  $\pm$  S. E. M.

## 5 DISCUSSION

As a result of the effect of alloxan, leading to diabetes, as evidenced from the significant ( $p < 0.05$ ) elevation in the level of blood glucose in rabbits (Figure 1), an observation which had also been reported by several workers [12], [13], [14], there is also derangements in protein metabolism in the plasma as well as in some selected tissues (liver, kidney and heart) which are implicated in diabetes.

Blood glucose level usually returns to normal within two hours after ingestion of carbohydrates in normal individual, but, in diabetes, blood glucose reaches a high level of above 115 mg/dL and remains elevated for longer period of time [15]. The hyperglycaemia status in the diabetic untreated rabbits may be attributable to deficiency of insulin which is responsible for the uptake of glucose from the blood. However, administrations of aqueous extract of *T. bangwensis* leaf and coconut water extract of *P. nitida* seed respectively decreased the blood glucose levels in diabetic rabbits demonstrating antihyperglycaemic properties. Aqueous infusions of some medicinal plants (such as *Eleophorbia druifera* and *Amaranthus sp.*) had earlier been reported to have antihyperglycaemic properties [16], [17]. It had also been reported that the leaf and stem of European mistletoe contain water soluble natural product(s) which directly stimulate insulin secretion from clonal B-cells [18]. This is also in agreement with the report of Gray and Flatt [6]. However, Coconut water extract of *P. nitida* seed also produced the desired normoglycaemia ( $\geq 75$  to  $\leq 115$  mg/dL) [15]. Possible hypoglycaemic effect had also been reported of aqueous extract of *P. nitida* seed [19]. Other probable mechanisms by which the extracts lowered blood glucose levels in diabetic rabbits might be by increasing glycogenesis, inhibiting gluconeogenesis in the liver, or inhibiting the absorption of glucose from the intestine [20]. The significant ( $p < 0.05$ ) reduction in the glucose level in the diabetic rabbit group placed on coconut water extract of *P. nitida* seed may in part be as a result of health benefits of coconut water which had earlier been documented to improve insulin secretion, improve utilization of blood glucose, being completely non-toxic and capable of relieving stress on pancreas [21]. The basis for the elevation of serum protein in diabetics is due to the fact that the serum is the medium through which the proteins and amino acids removed from the peripheral tissue are transported in the body [22]. However, the effects of insulin on protein metabolism (e. g. enhancement of amino acids uptake from the blood, protein synthesis and decreased protein degradation in tissue) is to decrease the level of protein in the serum [23]. When there is insulin deficiency as in diabetics, the reverse of the normal effects would occur and as shown in the present study (Figure 2) causing an increase in the serum protein level when compared with the control. It is possible that the hyperproteinaemia observed in this study may also be consequent upon damaged kidney and liver [24]. It is most likely that the test extracts which contain bioactive ingredients which possess insulin-like property which enhance normal protein metabolism.

Moreso, insulin inhibits proteolysis and vice versa in its absence. In insulin deficiency, glucose accumulates in the blood, hence, not absorbed by peripheral tissues thereby depriving cells of glucose for energy production and the body consequently, reverts to the use of protein for energy. In such situation, tissue proteins are degraded and the amino acids produced are used by the liver for energy production [15]. This may account for the reduction in protein level of the organs of the diabetic rabbits in this study (Figure 3). This is in agreement with the report of Zimmet *et al.* [25]. It is considered that

the test plant extract contain bioactive ingredients which tend to rehabilitate the protein levels in the selected organs under investigation. This may probably be by inhibiting gluconeogenesis and proteolysis in these organs thereby sparing protein for other metabolic activities instead of energy production.

## 6 CONCLUSION

The data generated from this study showed that diabetic rabbits were obtained using alloxan. The diabetic rabbits exhibited characteristics like hyperglycaemia and hyperproteinaemia. The extracts (aqueous extract of *T. bangwensis* leaf and coconut water extract of *P. nitida* seed) independently affected the diabetic rabbits as evident by the significant reduction in the levels of the serum and tissue biomolecules (blood glucose, protein) in the diabetic rabbits to almost the level in the control. Overall, the plant independently demonstrated to be efficacious as espoused by their antihyperglycaemic and hyperproteinaemic actions thus alleviating the biochemical disorders associated with diabetes and hence confirming the antidiabetic activity of the extracts.

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