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Effect of aqueous extract of *Tapinanthus bangwensis* on serum electrolytes in alloxan-induced diabetic Wistar Rats

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Abstract

This study investigated the effect of aqueous extract of *Tapinanthus bangwensis* on some serum electrolytes in alloxan-induced diabetic rats. The effect of this plant extract was monitored on the serum concentration of potassium iron (k^+), sodium ion (Na^+), uric acid, bicarbonate (HCO_3^-), and urea. Sixty-six albino rats were used for the study arranged into eleven groups of 6 rats each. Two groups (12 rats) were used for the pilot study. Nine other groups (54 rats) were used for the experiment labelled groups 1-9. Group 1 constitute the normal control which received only feed and water, group 2 received 50mg/kg citrate buffer. Group 3 was administered alloxan solution and allowed free access to feed and water. Group 4-6 received 50mg/kg of citrate buffer and were also administered with the aqueous extract of *Tapinanthus bangwensis* at a dose of 250mg/kg and referred to as normal treated, concentration 1(NT conc-1), concentration 2 (NT con-2) and concentration 3 (NT conc-3) respectively. Groups 7-9 were administered 50mg/kg alloxan and different grades (5%, 7%, 10%) respectively of the aqueous extract. Blood liver and pancreatic tissues were collected into appropriately labelled sample bottles and analyzed. The result of the blood analysis showed electrolyte levels were only slightly altered with potassium ions having the least change. Bicarbonate rose from 22.67±1.15 to 24.50±1.52, potassium ions decreased from 4.45±0.14 to 4.43±0.22 and sodium ions rose from 139.33±1.63 to 143.00±2.90. The metabolite uric acid was elevated from 2.75±0.12 to 3.35±0.33. Oral administration of 250mg/kg aqueous *Tapinanthus bangwensis* extract to groups 7,8 and 9 of 50mg/kg, 70mg/kg, and 100mg/kg respectively, significantly decreased many of these biochemical alterations in a dose-dependent manner. 100mg/kg extract showed the highest effect in lowering the elevated parameters followed by 70mg/kg administration. The 50mg/kg dose had the least lowering effect. Histopathological results also confirmed these chemical pathological results. The extract of *Tapinanthus bangwensis* is insulinogenic and thus can be a good antibiotic agent as it can improve most of the altered biochemical and physiological parameters observed during diabetes mellitus.

Keywords: *Tapinanthus bangwensis*; Serum electrolytes; Citrate buffer; Diabetic treated concentrations; Alloxan

1. Introduction

In recent times, an endless search by man for substances or agents with medical values has gathered much momentum towards plants and plant products because of their little or no side effect (Prout, 1974) and their rich medicinal values (Obi *et al.*, 1998).

Many of the plant species growing throughout the world have medicinal values with active constituents that have a direct action on the body (Uahomo et al., 2022). They are used both in herbal and conventional medicine and offer benefits that pharmaceutical often lack helping to combat illness and support the bodies effort to regain good health (Singh *et al.*, 2007).

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Odia and Wokoma, (1992) have stated that diabetes is the leading cause of morbidity and mortality globally. Between 1980 and 2004, diabetic Americans rose from 5.8-94.7 million with 1.4 million cases recorded in 2004 alone within the ages of 18-79 years old. This number is expected to increase in 2005 (Cusick *et al*., 2005). The known risk factors of diabetes include genetic susceptibility, sedimentary lifestyle, obesity, hyper-insulinaemia etc. (Warram and Krolewski, 2005) and cigarette smoking (Kim *et al*., 1995).

A normal pancreas secretes everyday 2-2.5 litres of bicarbonate- rich fluid containing digestive enzymes and proenzymes. In addition to digestive enzymes, the pancreas secretes water and bicarbonate stimulated by the hormones, secretin and cholecystokinin. Secretin stimulates water and bicarbonate production by the duct cells. The lining epithelial cell of the pancreatic duct secretes electrolytes. The pancreas is thus an important organ involved in the metabolism of various substances in the body such as in the secretion of the electrolytes.

In recent times, Omeodu and his colleagues have been actively carrying out research with different plant extracts on diabetic models to proffer possible plant based management measures for diabetes (Omeodu et al., 2023; Omeodu et al., 2022; Omeodu et al., 2022; Akoko et al., 2022).

This current research investigates the effect of aqueous extract of *Tapinantus bangwensis* on alloxan induced diabetic wistar albino rats. The purpose is to find out if *Tapinathus bangwensis* which has been known to have anti-diabetic effects (Obatomi et al., 1994; Swason-flatt *et al*., 1989) could lower biochemical parameters like serum electrolytes and uric acid.

2. Material and methods

2.1. Preparation of plant materials

The plant used for this work is *Tapinathus bangwensis*. The plant was found in the University of Port Harcourt where it was found hemi-parasitizing on specie of orange (*Citrus aurantium*) orchard located on the right side of the front of the Vice Chancellors lodge at the Delta Park of the university. The leaves which were used for this work were carefully plucked off, thoroughly washed and air dried for 24 days until a constant weight was obtained.

2.2. Preparation of aqueous *Tapinanthus bangwensis*

Tapinathus bangwensis was collected with stalks. The fresh greenish leaves were carefully plucked off from the stalks and pedicle removed from each leaf. The leaves were thoroughly washed and spread out on a clean cardboard paper and kept at room temperature in a well aerated room. They were allowed to dry to constant weight after 24days. The dried sample was then pounded in a mortar with pistil. After pounding, the partially powdered sample was grounded in a manual grinding machine until a fine powder was obtained. Fifty grams of the powdered mistletoe was measured and dissolved in a 1 L measuring cylinder containing 500ml distilled water. The mixture was thoroughly shaken for 10 minutes. The mixture was then stored at room temperature for twenty-four hours (Omoedu et al, 2008). The preparation was filtered using ten different pieces of white cloth. The filtrate was filtered two times through a Whatman No. 541 filter paper and stock was stored in a refrigerator at a temperature of 40^{o}C for 24 hours. 50mg/kg, 70mg/kg and 100mg/kg of the filtrate were then prepared from the stock solution and these three different concentrations were used to treat the test animals (Omoedu et al., 2008).

2.3. Experimental protocol

The animals were divided into experimental groups of six (6) animals per group and each group was housed in a metabolic cage. They were provided with feeds and water ad libitum. The animal feeds were purchased from the livestock feeds, Choba, a division of Livestock Feeds Nig. Ltd. Ikeja, Lagos while the water was supplied by the water treatment plant, Choba Park, University of Port Hracourt. There was a total of (9) experimental group. All the rats weighed between 200g-300g and their average age was fourteen (14) months.

The investigated animals consisted of nine groups with six animals per group (the experimental group is shown below). Each animal was labelled with picric acid for easy identification on the head (HD), right hands (RH), right leg (RL), left hands (LH), left leg (LL) and tail (TL).

Group one animals were administered only feeds and water *ad libitim to* serve as general control group. Group two animals received citrate buffer solution in addition to feeds and water. Alloxan solution was administered to group three animals and allowed free access to feed and water. Before citrate and alloxan administration to group two and three respectively, the animals were fasted for 18 hours. This was the same for group 4 to 9 animals that received various

treatments. Group four to six were administered with citrate buffer at 50mg/kg dose, while groups seven to nine were administered with alloxan solution at same 50mg/kg and then treated with *Tapinanthus bangwensis* solution at a dose of 250mg/kg with group seven receiving 50mg/kg of the *Tapinanthus bangwensis* extract, group eight receiving 70mg/kg and group nine 100mg/kg of the extract.

Table 1 Research Design

Groups	Identification	No of Rats	Treatment
Group 1	Normal control 1 (NC-1)	6	Feed + H_2O Only *ad libitum*
Group 2	Normal control 2 (NC-2)	6	Feed + H_2O *ad libitum* + citrate buffer
Group 3	Normal diabetic control (NDC)	6	Feed + H_2O *ad libitum* + alloxan solution
Group 4	Normal treated control (NT-1)	6	Feed + H_2O *ad libitum* + citrate buffer 5% mistletoe solution
Group 5	Normal treated control (NT-2)	6	Feed + H_2O *ad libitum* + citrate buffer + 7% mistletoe solution
Group 6	Normal treated control (NT-3)	6	Feed + H_2O *ad libitum* + citrate buffer 10% mistletoe solution
Group 7	Diabetic treated control (DT-1)	6	feed + water *ad libitum* + alloxan + 5% mistletoe solution
Group 8	Diabetic treated control 2 (DT-2)	6	Feed + water *ad libitum* +alloxan + 7% mistletoe solution
Group 9	Diabetic treated control 3 (DT-3)	6	Feed + water *ad libitum*+ alloxan solution+ 10% mistletoe solution.

2.4. Administration of *Tapinanthus bangwensis* extract

The *Tapinanthus bangwensis* solution was prepared into 5%, 7% and 10% by the process already stated by Omoedu et al., (2008). These three different preparations were fed only to groups 4, 5 and 6 respectively at a dose of 250mg/kg body weight of animal on daily basis. The treatment continued for twenty-one (21) days at the end of which all the nine groups were sacrificed by cervical dislocation method and their whole blood collected for analyses. Each of the animal's pancreas and liver were also collected and preserved in 10% formaldehyde.

2.5. Sample collection for analyses

At the end of the twenty-one days of extract administration, the animals were sacrificed on the twenty second day. Each rat to be sacrificed was withdrawn from the cage and anaesthetized in chloroform saturated chamber (Omoedu et al., 2008). The blood sample was then collected from the animal after withdrawing from the chamber by cardiac puncture into appropriately labelled sample bottles, its pancreas and liver tissues were also collected into separate sample bottles and preserved in formaldehyde. These samples at the end of collection were quickly taken to the laboratory for analyses. The blood specimen was centrifuged at 5000rpm using MSE centrifuge to obtain plasma. The liver and pancreatic samples were prepared into slides and analysed at the anatomical histopathology, laboratory of the University of Port Harcourt Teaching Hospital.

2.6. Estimation of serum uric acid

Uric acid was converted to allantoin and hydrogen peroxide by uricase. The hydrogen peroxide formed was reacted with 3,5-dichloro-2-hydroxybenzene sulphonic acid and 4-aminophenazone to produce a red violet coloured N-(4-antipyryl)-3-chloro-5-sulphonate -p-benzo-quinoneimine. The reaction mixture was incubated for 5 minutes at 37^0C and absorbance read at 520nm.

2.7. Estimation of serum sodium (Na), bicarbonate (HCO_3^-) and potassium (K) ions

The determination of these electrolytes in serum was carried out by the use of ion selective electrode which is an automated machine with the application of sensor and micro-computer technology. The manometric method was used for the determination of sodium bicarbonate (HCO_3^-) in the test sample. Both the serum sample and reagent were added into the sealed reaction chamber. As the reaction proceeds, the bicarbonate ions present in the sample released carbon (iv) oxide (CO_2) gas and the evolution of the gas increases the pressure inside the reaction chamber and the pressure sensor detects the changes and sends the signals to the microprocessor which determines the amount of HCO_3^- in the serum. The machine displays this value and prints out the data.

2.8. Data Analysis

The data was statistically analyzed using GraphPad Prism Software (2000) version 3.05 by GraphPad Inc. Data are presented in Mean±Std. Statistical significance was accepted at a level of $p<0.05$ and below. Analyzed data were presented in charts.

3. Results

The results of the investigations shown on Figures 1,2,3,4 and 5 indicated clearly that alloxan caused diabetes mellitus in the experimental animals. Values of the control animals were found to be within normal range for the parameters analysed.

Figures 1 and 2 illustrate the effect of the treatment of alloxan-induced diabetic rat with aqueous mistletoe extract. Serum bicarbonate ions showed marked decrease on treatment of the alloxan-induced diabetic rats with *Tapinanthus bangwensis* extract. Figures 1 and 3 illustrate the effect of aqueous *Tapinanthus bangwensis* extract on alloxan induced diabetes and serum potassium ion levels. The effect treatment of the plant extract on alloxan-induced diabetes and the serum ion levels in the experimental animals is shown on figures 1 and 4. There was no significant effect on serum sodium ions observed. Figure 5 shows the effect of treatment with aqueous extract of *Tapinanthus bangwensis* on serum uric acid levels of alloxan-induced diabetic rats.

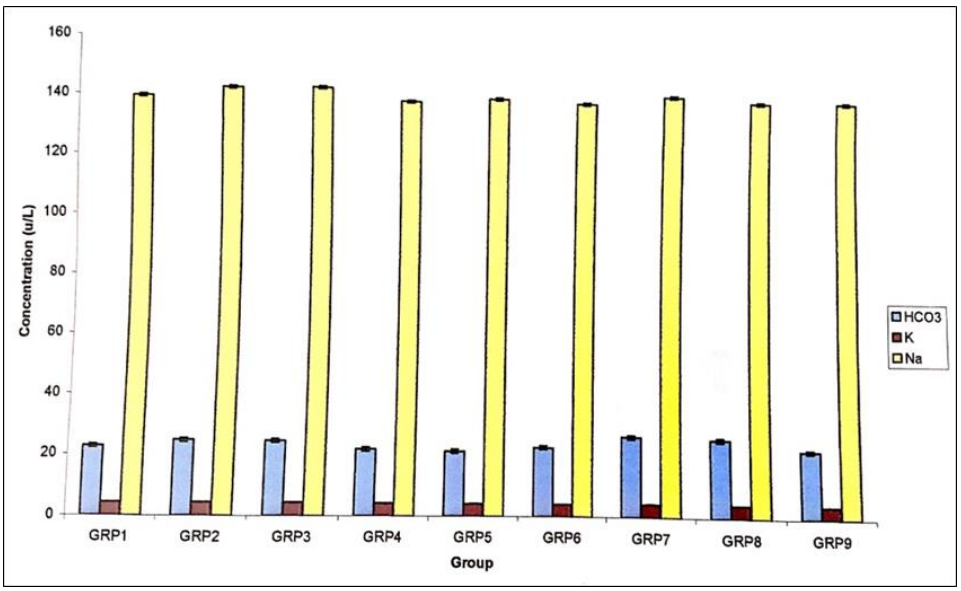

Figure 1 Concentration versus experimental groups of electrolytes in both non diabetic and diabetic rat

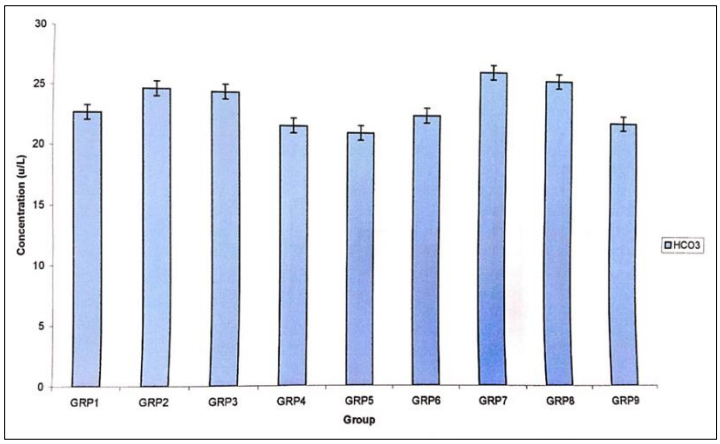

Figure 2 Concentration versus experimental groups and their serum bicarbonate ion level

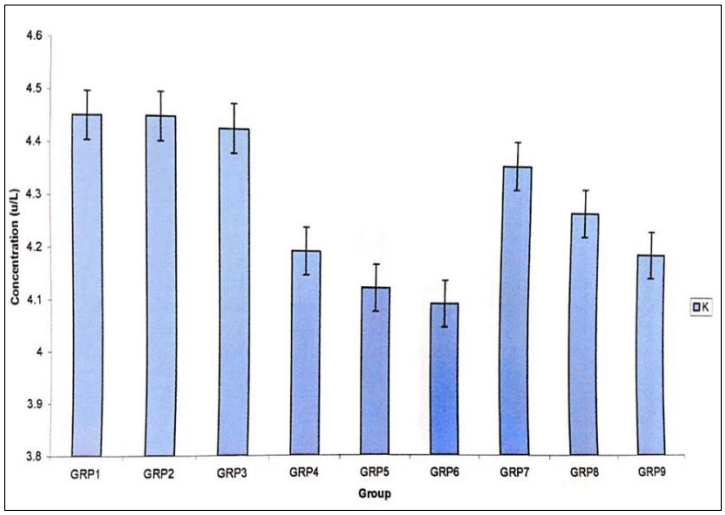

Figure 3 Concentration versus experimental groups and their serum potassium ion level

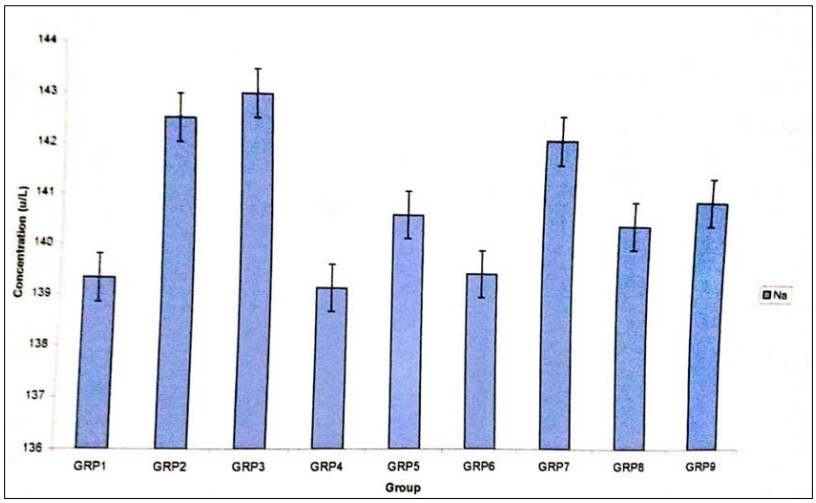

Figure 4 Concentration experimental group and their serum sodium ion level

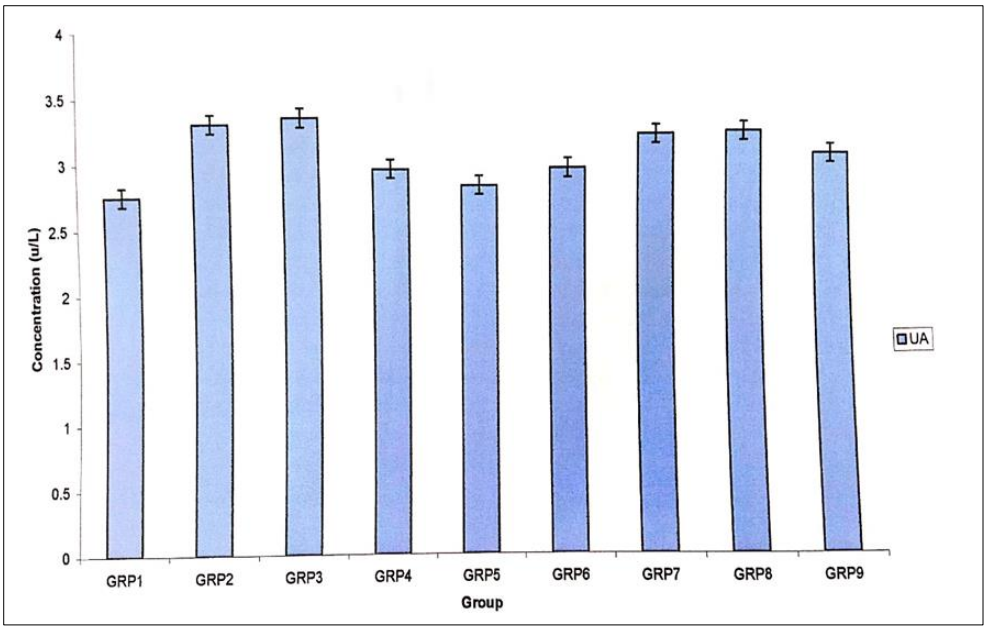

Figure 5 Concentration versus experimental groups and their serum uric acid ion levels

3.1. Effect of *Tapinanthus bangwensis* on the liver and pancreas of diabetic rats

Morphological observation of the liver of group 3 animals shows vacuolated cytoplasm, lesions and a degenerative cytoplasm while those of groups 4-6 show normal architecture and groups 7-9 shows a gradual return to normal liver architecture in a dose dependent manner with group 7 having a close resemblance to group 3 with massive area of necrosis and group 9 having similar architecture to normal liver architecture of groups 1 and 2 animals.

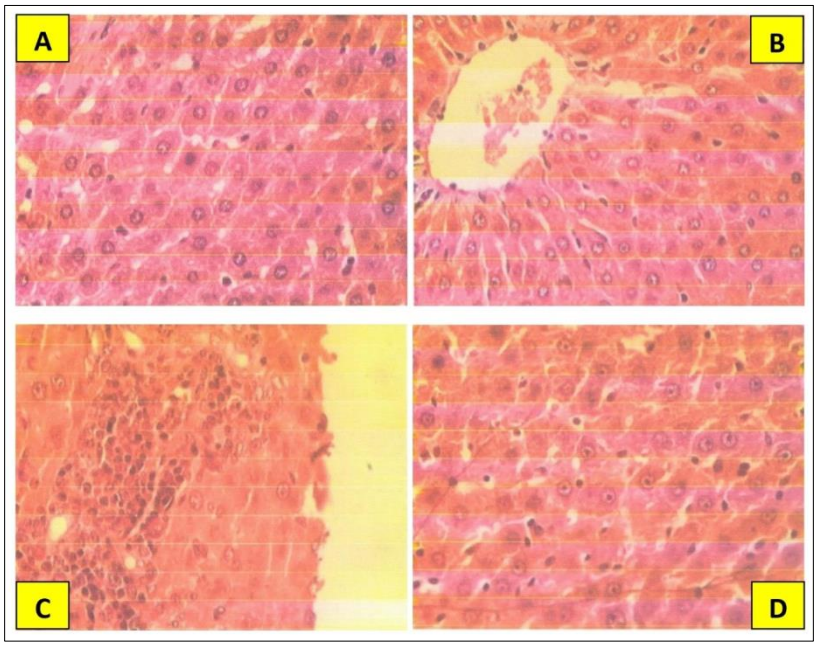

Figure 6 Photomicrograph of the Liver. (A) Histology of the liver showing a normal liver architecture; (B) Histology of the liver showing a vacuolated cytoplasm with degenerative Kupffer cell; (C) Histology of the liver showing massive area of necrosis with degenerative cytoplasm; (D) Histology of the liver showing only few areas of necrosis. Magnification: x200

Morphological observation of the pancreas of the experimental animals also shows group 3 animals having extensive necrosis of islets and reduced number of cells. Group 4 -6 have architecture close to the normal groups 1 and 2. While

groups 7 -9 shows reduction in necrosis of the islets in a dose dependent manner with group 7 (5% extract) having more necrosis than groups 8 and 9 administered with 7 and 10% extract respectively.

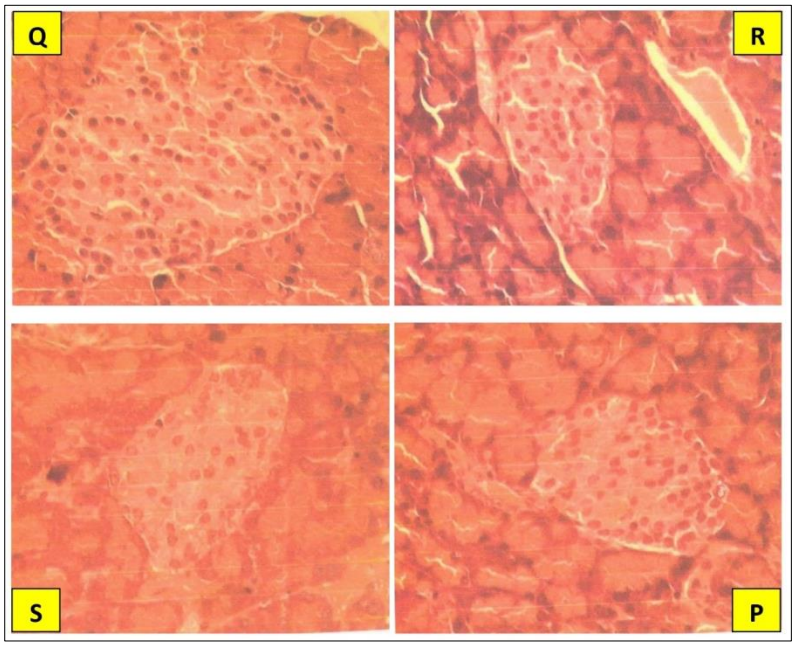

Figure 7 Photomicrograph of the Pancreas. (Q) Histology of the pancreas showing normal pancreas architecture with normal islets; (R) Histology of the pancreas showing extensive necrosis of islets with reduction in number of cells; (S) Histology of the pancreas showing enlarged islet cells (hyperplasia) with patchy necrosis; (P) Histology of the pancreas showing patchy necrosis with few inflammatory cells. Magnification: x200

4. Discussion

Several researchers on African medicinal plants have reported significant success with minimal or no side effects (Prout, 1974). Swaston-Flatt *et al*. (1989) reported that many clinical parameters associated with diabetes mellitus in experimental animals were reduced when administered with the extract of *Tapinanthus bangwensis* (6.25%) by weight. He observed that such diabetes -associated symptoms as polydipsia, polyhagia and body weights loss were all ameliorated with mistletoe administration. The works of Diden *et al.,* (2005) and Obatomi et al. (1994) have lent credence to this present work. In this work, it was observed that alloxan given to experimental rats at a dose of 50mg/kg induced diabetes mellitus in them. Diabetes was not observed when the carrier solvent citrate buffer administered to non-diabetic rats, hence the diabetogenicity was caused by only alloxan. Subsequent administration of aqueous extract of *Tapinanthus bangwensis* to the experimental groups (7,8 and 9) with 50mg,70mg and 100mg per kg ameliorated the diabetogenic effect of alloxan. Treatment of extract of *Tapinanthus bangwensis* on the diabetic rats did not have only significant difference on the serum electrolytes studies in this work Na^+, K^+, and HCO^{3-}. Totan and Greaby (2002) have investigated and reported that red cell Na^+, K^+ ATPase plays a vital role in the regulation of cationic haemostasis and in an altered state of NA^+, K^+ ion concentration during complications of diabetes mellitus. Thus, this helps the electrolyte balance in diabetics (Syed *et al*., 1994). The mechanism thus ensures little or no change in the serum levels of Na^+, K^+ and HCO^{3-}. Hyperuricaemia was observed in this study after treatment with alloxan. This situation has been reported by Cappacio *et al. (*1993) where he observed high uric acid levels in insulin resistant diabetic patients, this study was confirmed by Dehghan *et al*. (2008). Extract administration to the test animals showed some slight reduction to the acid levels in a dose-dependent manner. After treatment with alloxan, there may be some surviving β-cells and regeneration is also possible (Gomes *et al.*, 2001). This study was therefore able to establish the diabetogenicity of alloxan as seen in the glucose level, and that in diabetes mellitus, electrolytes and uric acid, concentration is elevated.

5. Conclusion

This work has shown that extract of *Tapinanthus bangwensis* is insulinogenic and thus can be a good antibiotic agent as it can improve most of the altered biochemical and physiological parameters observed during diabetes mellitus.

Compliance with ethical standards

Acknowledgments

The authors wish to acknowledge and appreciation all laboratory assistants who provided one assistance or the other to ensure that this study was a success.

Disclosure of conflict of interest

The authors declared that no conflict of interest exist.

Statement of ethical approval

All procedures carried out during this study were done in accordance with the guiding principles of research involving animals as recognized by the Research Ethics Committee of the University of Port Harcourt.

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